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## Report of the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM)

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Hamburg, Germany

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International Council for the Exploration of the Sea  

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## Executive summary

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The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met in Hamburg, Germany from 3 to 5 May, 2004. Fifteen persons representing ten countries were present with five others participating through correspondence prior to and during the meeting. Five terms of reference (ToR) were addressed, although one was dismissed as the appropriate information was unavailable for review.

The WG first considered ToR a: Provide recommendations on the applications for the estimation of effective population size in wild populations of marine fish and shellfish. In this ToR we critically consider the methods used to estimate effective population size, together with their technical and statistical limitations. We then provide a brief review of current studies on marine species and identify the range of factors that may influence  $N_e$ . Finally, we consider the implications of current patterns and suggest recommendations in relation to data quality and fisheries management. This ToR should provide valuable background for those wishing to calculate or interpret data on effective population size. The WG recommended that:

- 1) More studies are needed among and within species where population genetic data, demography and life-history traits are well documented to determine the generality of the  $N_e/N$  patterns observed so far. Such studies should include considerations such as temporal stability as well as population and species specific heterogeneity.
- 2) In order to assess the relative contribution of historical and contemporary processes on  $N_e/N$  ratios, additional studies that assess long term variance in  $N_e$  estimates (e.g. using coalescence theory) are required.
- 3) Prior to undertaking analysis it is crucial to design a sampling programme in accordance with the underlying assumptions necessary for generating precise  $N_e$  estimates (e.g. that the population is relatively closed and without significant substructure).
- 4) In addition to optimal sampling design, it is imperative to exercise sound quality control of genetic data (e.g. genotyping consistency) prior to statistical analysis.
- 5) It is recommended that estimation methods are validated through simulation and theoretical studies, in order to quantify the contribution of genetic marker variability and technical artefacts, such as null alleles, mis-scoring, large allele drop out, on  $N_e$  estimates, and to identify the limitations of the available methods.
- 6)  $N_e$  and  $N_e/N$  estimates and their long term trends should be examined over successive temporal sampling dates and across generations to test signal-to-noise ratios.
- 7) To address the uncertainty inherent in fulfilling the assumptions needed for precise  $N_e$  estimation it is recommended that independent methods are employed simultaneously to test for concordance.
- 8) Significant disparities between  $N_e$  and  $N$  should be considered in stock assessment models to reduce uncertainty in recruitment relationships.

For the second consecutive year the WG was asked to consider ToR b: *Evaluate the management recommendations for Atlantic salmon, developed by the SALGEN EU project*. SALGEN is an EU project set up to review genetic studies on Atlantic salmon and develop management recommendations for the species. It was expected that the project topic reviews and management recommendations would be published in book form in early 2004. However the project has encountered some production delays. Unfortunately as a consequence WGAGFM have been unable to undertake the envisaged review at this time. It is recommended that the review of SALGEN project outputs be removed from the WGAGFM terms of reference.

The European eel has a complex life history and has for a long time been assumed to form a single panmictic population. Although never observed, spawning occurs in the Sargasso Sea, with larvae moving eastwards to the continental shelf of Europe where, after metamorphoses, they majority ascend the rivers of Europe and North Africa, eventually returning to the Sargasso Sea as adults. Recent population genetic studies DNA fingerprinting with microsatellite markers aimed to solve the population structure in eel. The WG considered conservation genetic aspects of the European eel in ToR c: *Consider conservation genetics aspects required for conservation targets for eels*. Summarizing the available knowledge the WG concluded that the genetic structure of European eel is still inconclusive. Mitochondrial haplotypes, allozymes and microsatellites show high levels of genetic diversity, pointing to the long evolutionary history of European eel and the large population sizes. Genetic differences between geographic regions are present but have not yet been confirmed to be stable in time. Isolation by distance of several spawning populations is only supported by a two studies lacking temporal replication. The view of the WG is that temporal genetic variation may play a significant role in explaining the spatial structure reported earlier for this species. The WG advanced 7 recommendations for this ToR which are contextualized in this report:

- 1) It is recommended that work is undertaken to delineate the spawning grounds;
- 2) Eel fisheries management should be conscious of subtle genetic structure and follow the precautionary principle;
- 3) It is recommended here that the precautionary principle be adopted to protect as of yet unresolved genetic variability, and as a consequence the transfer of glass eels between basins should be avoided;
- 4) We recommend that sufficient adult recruitment is ensured to maintain a large and spatially representative silver eel population;
- 5) It is recommended that support be given to current EIFAC/ICES initiatives to collect better spawning stock size data;
- 6) It is recommended that an inventory of European eel otolith archives be made. This database should include information regarding the condition of the samples in the archive and methods used to preserve the samples;
- 7) Information on the sex composition of migrating silver eels should be routinely collected in ongoing and future monitoring programmes.

Probabilistic maturation reaction norms and their use in detecting a genetic impact of selective fishing formed a theme session at the 2002 ICES ASC. To date, they have been applied to 11 marine stocks and 1 freshwater stock of 4 species (cod, plaice, herring and grayling). The WG reviewed and evaluated this method in ToR d: Evaluate the use of reaction norms to evaluate the genetic impact of selective fishing. The WG endorsed the use of probabilistic maturation reaction norms as a tool for evaluating the evolutionary and genetic effects of selective fishing and recommended additional scientific investigations on:

- a) assessing fisheries-induced evolution in adaptive traits other than maturation, including growth rates, reproductive effort, skipped spawning, and behaviour;
- b) measuring quantitative genetics parameters of exploited stocks needed for predicting the expected pace of fisheries-induced evolution;
- c) clarifying the mechanisms that link fisheries-induced evolution to decreases in stock stability, yield, and recovery potential;
- d) investigating how fisheries regimes that are least detrimental for genetic composition depend on the life-history patterns of exploited stocks;
- e) developing appropriate management and assessment tools for coping with fisheries-induced evolution.

The WG also recommended that ICES sponsor an international symposium on fisheries-induced evolution. Such an event will serve as a platform for integrating recent scientific advances, facilitating the lines of future research summarized above and in our report, and for initiating development of the practical tools that will be needed in the future for the assessment and management of fisheries-induced evolution.

Lastly, the WG was given a ToR through the WGECO (ToR e): Commence work on a list of species for which there is reason to be concerned for loss of genetic variation, and a list of species for which there is good genetic information from which to advance management advice. In compiling this information the WG only included data on commercial marine species from the North Atlantic and adjacent seas, and only those species where structuring has been detected and where we felt that there was a reliable case supporting the subdivision(s). Twenty-one commercial species were identified (with supporting evidence) as good candidates for commencing the process of developing advisory forms incorporating the preservation of genetic diversity. It is assumed that this list will be provided to WGECO for the next steps in this process. Concerns were raised over the lack of study on the genetic impacts of bycatch of target and non-target species and the need for long term time series to track changes in genetic diversity. In addition to the requested list, two recommendations were made:

- 1) *Historical collections of commercial and other species are of extreme value in assessing loss of genetic diversity due to anthropogenic effects. These should be given a high priority for preservation and made available for genetic research.*
- 2) *Careful analysis of genetic and phenotypic variation should be made and integrated into physical oceanographic and ecological knowledge and modelling in stock assessments.*

In concluding its business the WG proposes to meet at the Danish Institute for Fisheries Research in Silkeborg, Denmark in 2005.

# 1 Introduction

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The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) met at the Federal Research Centre for Fisheries, Institute for Fishery Ecology, Hamburg, Germany, 3–5 May 2004 to deal with its Terms of Reference (ToRs) for 2004 (Annex 1), with E. Kenchington (Canada) as the Chair. The ToRs were decided in Council Resolutions (C.Res. 2003/2F04, Annex 1) adopted at the Statutory Meeting held in Tallinn, Estonia, 2003. The meeting was opened at 9:00 h on Monday, 3 May, with the Chair welcoming the participants. This was followed by a welcome from Professor and Director Hans Jenke of the Institute for Fishery Ecology and our German Host, J. Trautner.

## 1.1 Attendance

Fifteen persons representing ten countries attended the 2004 WGAGFM meeting (Annex 2). Apologies were accepted from E. Eg Nielsen and M. Møller Hansen (Denmark), P. Prodohl (UK, N. Ireland), A. Danielsdóttir (Iceland), J.-M. Sévigny (Canada), S. Fevolden (Norway), R. Wenne (Poland), S. Stiles (USA), and T. Paaver (Estonia). Five others (H. Wilcock, M. Heino, B. Hutchinson, M. M. Hansen and E. E. Nielsen), including two Working Group (WG) members contributed substantially to the meeting intersessionally through correspondence, but were unable to attend.

Attendance was greatly improved over the previous year and the WG felt that this could be attributed to the 2003 ICES survey of members to confirm their participation, and to moving the meeting date to May when the academic year was over, as many of our members have university affiliations. The WG was greatly complemented by the participation (by invitation) of U. Dieckmann (Austria) and B. Ernande (France) who provided very interesting presentations on probabilistic maturation reaction norms for our consideration. The WG would also like to acknowledge the assistance of students K. Roth and K. Schmidt (Germany) who participated in the meeting.

## 1.2 Venue

The Federal Research Centre for Fisheries lab at Hamburg (represented by our host J. Trautner) did an excellent job of arranging logistics and facilities for the meeting, including a group dinner on Tuesday evening sponsored by the Federal Ministry of Consumer Protection, Food and Agriculture. The meeting in Hamburg was held in a fully equipped meeting room which greatly enhanced our working environment. The WG wishes to thank Jochen for all of the work undertaken to arrange this meeting and for his very kind hospitality.

## 1.3 Meeting format

WGAGFM has an established framework for completing its Terms of Reference (ToRs). Prior to the meeting, small *ad hoc* working groups, under the leadership of one person, are established to prepare position papers related to specific issues in the ToRs. The leader of the ToR is responsible for presenting the position paper in plenary at the meeting and chairing the discussion. Thereafter, volunteers undertake the task of editing and updating position papers according to points raised in the plenary discussions. The ToR leader is responsible for preparing the final report text from their sessions. All reports and recommendations are reviewed in plenary to achieve a consensus from those present on the final wording.

Unfortunately, due to various circumstances, ToR leaders did not follow this process this year and no position papers were ready for presentation at the start of the meeting. However, most ToRs were sufficiently advanced that they could be brought to a state of completion relatively quickly. The WG worked interactively to prepare the report during the meeting with ToR leaders meeting in plenary for group input and returning to smaller working groups to incorporate comments. This worked reasonably well and allowed for the ToR to be fully addressed through the course of the meeting. Only ToR a) required correspondence after the meeting in order to finalize the text. However, all recommendations were reviewed and agreed upon in plenary.

It was agreed that the Chair would be very clear to members that in proposing ToR for 2005, leaders must declare their intent to follow the WG framework and to make a reasonable effort to attend the 2005 meeting. We propose to commence the 2005 meeting with presentations by the WG leaders at which time a draft response to the ToR will be circulated. Discussion and input from the WG will be given through the course of each presentation and the remainder of the meeting will be spent incorporating the comments of the WG into the ToR response with meetings in plenary as required to finalize the documents. This will allow a greater participation by the full membership in the responses to all ToRs. We also propose to extend the length of the meeting by one day to ensure adequate time to address the complex ToRs we are dealing with (see Section 3.1 below).

The 2004 WGAGFM meeting proceeded under the following direction:

- E. Kenchington chaired business and general scientific sessions;

- D. Bekkevold led ToR a: Provide recommendations on the applications for the estimation of effective population size in wild populations of marine fish and shellfish;
- P. McGinnity led ToR b: Evaluate the management recommendations for Atlantic salmon, developed by the SALGEN EU project;
- J. Trautner led ToR c: Consider conservation genetics aspects required for conservation targets for eels;
- U. Dieckmann and B. Ernande led ToR d: Evaluate the use of reaction norms to evaluate the genetic impact of selective fishing;
- E. Kenchington and G. Dahle led ToR e: Commence work on a list of species for which there is reason to be concerned for loss of genetic variation, and a list of species for which there is good genetic information from which to advance management advice.

## 2 Terms of Reference for 2004

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### 2.1 Provide recommendations on the applications for the estimation of effective population size in wild populations of marine fish and shellfish (ToR a)

This text was based on a position paper prepared by D. Bekkevold, G. Carvalho, M.-L. Koljonen, M. M. Hansen, E. E. Nielsen, B. Hutchinson and H. Wilcock and adopted by WGAGFM in Hamburg in 2004.

#### 2.1.1 Introduction: Relevance of $N_e$ to fisheries biology and management

An estimation of the size of a fishable resource is crucial if it is to be managed effectively. Such information provides a basis for the formulation of stock abundance measures, such as census population sizes (“head-count” of individuals present) and spawning stock biomass (accounting for year classes, age at maturity and the length and weight of the spawning fish); key parameters used in defining appropriate levels of exploitation. For most marine finfish species, census and spawning stock estimates have often suggested that stocks retain large population sizes despite intensive exploitation. This has been one of the primary arguments behind the continuation of fishing on heavily depleted stocks. Of the total census population size ( $N$ ), however, only a proportion (the effective population size  $N_e$ ) will pass on their genetic characteristics to the next generation in any one reproductive bout. Two mathematicians, Sir Ronald Fisher (UK) and Professor Sewall Wright (USA), laid the groundwork for estimation of  $N_e$  in the 1930s. Importantly they made a connection between the magnitude of random change in populations between generations (genetic drift) and the number of parents that were successful in leaving offspring. A large number of successful parents would limit the amount of genetic drift, and vice versa. Thus, population genetic theory can be used to “count” the number of fish actively reproducing at any one time: the amount of drift that is occurring can provide an indirect measure of the number of spawning fish in a population that successfully leave offspring (recruits) in the next generation.

Until recently, it was generally believed that the majority of wild marine fish populations were sufficiently large to be unaffected by random change between generations (drift) during population declines, and that population connectivity for most species was generally high. Indeed, much previous genetic work (reviewed by Carvalho, 1998; Ward, 2003) supported such assertions, with marine fishes showing usually higher levels of genetic variability and lower levels of genetic differentiation than freshwater or anadromous species (Ward *et al.*, 1994). Although earlier work had suggested that some marine species might have considerably lower effective population sizes ( $N_e$ ) than census sizes (Hedgecock *et al.*, 1989; 1992; 1994) due to marked variance in reproductive success, it is only within the past few years that new genetic methods have been employed to provide estimates of  $N_e$  in wild marine fish populations. Recent data (Hauser *et al.*, 2002; Turner *et al.*, 2002; Hutchinson *et al.*, 2003) now indicate that  $N_e$  in marine fishes, especially those characterised by high fecundity and high larval mortality, is typically  $10^2$  to  $10^6$  orders of magnitude smaller than census population sizes. Such discrepancies have profound implications for estimating both quantitative change in population size relative to recruitment and harvesting, but also for qualitative change, in terms of the nature and speed of genetic change in marine populations. A low ratio of effective population size to census size ( $N_e/N$ ), suggests greater vulnerability to changes in genetic diversity, patterns of genetic differentiation and responses to environmental change (selection pressures). The relationship between successful spawners and subsequent recruits is critically important to stock assessment models. Genetic estimates of effective population size may assist in the formulation of more accurate, independent measures of these parameters.

In this ToR we will critically consider the methods used to estimate effective population size, together with their technical and statistical limitations. We then provide a brief review of current studies on marine species and identify the range of factors that may influence  $N_e$ . Finally, we consider the implications of current patterns and suggest recommendations in relation to data quality and fisheries management.



### 2.1.2 Estimating effective population size

Molecular markers, primarily microsatellites and mtDNA are being applied increasingly for estimating effective population size. Here, we focus on methods for analysing microsatellite markers, although mtDNA has proven very useful for estimating long-term  $N_e$ , e.g. using approaches based on coalescence theory, and for inferring long-term demographic patterns using mismatch distribution analysis.

Basically, there are two fundamentally different types of methods for investigating  $N_e$ :

- Methods for directly estimating effective population size ( $N_e$ )
- Methods for detecting changes in  $N_e$  without actually providing an estimate of  $N_e$ . These methods will not be considered further in this context, though it should be pointed out that some of them have proven really useful, in particular that of Garza and Williamson (2002).

#### 2.1.2.1 Temporal methods

The so-called temporal method has proven to be the most useful method for estimating  $N_e$  (e.g., Waples 1989; Jorde and Ryman 1995). The basic principle is to sample a population at two or more points in time separated by a specified number of generations. The smaller the  $N_e$ , the more random genetic drift will occur. Thus, based on the random changes in allele frequencies that have occurred during the interval it is possible to estimate the effective population size.

The first methods developed were based on estimating  $N_e$  from the standardized variance of allele frequencies among temporal samples (see Waples 1989 for an overview). The temporal method assumes discrete generations, as well as samples drawn randomly from the entire generation. In species with overlapping generations and with samples biased towards specific age classes, temporal changes in allele frequencies are dependent not only on  $N_e$  but also genetic differences between cohorts (Jorde and Ryman 1995). This downward bias in  $N_e$  becomes smaller with increasing time between sampling occasions, as the contribution of genetic drift to temporal shift in allele frequencies relative to differences between cohorts increases with the number of generations between sampling occasions. While it is therefore beneficial to use generations that are further apart, the calculated  $N_e$  will represent an average ('harmonic mean') over all the intervening generations, including some which have a strongly biased recruitment success, or higher than average mortality, and some that have more equal survivorship.

A number of temporal methods have been developed employing maximum-likelihood, Markov Chain Monte Carlo re-sampling, Bayesian and/or coalescence approaches. The main advantages of these methods are that they make better use of the whole data set compared to the original method based on the standardized variance of allele frequencies. Also, Bayesian methods allow for incorporating prior information, such as putting a realistic upper limit on  $N_e$  and thereby avoiding estimates that are undefined (i.e.  $N_e$  is "infinitely" high). Finally, coalescent models provide a good approximation to the case of overlapping generations.

One very useful temporal method is that originally proposed by Berthier *et al.* (2002) and further developed by Beaumont (2003). The method is Bayesian and coalescence-based and allows for putting a prior upper limit on  $N_e$ . Furthermore, the latest version allows for inclusion of several (i.e. more than two) temporal samples. It can be used both for estimating the harmonic mean of  $N_e$  over the entire time span covered by the samples, and for estimating  $N_e$  at the beginning and end of the time interval, thereby providing information on whether the population is expanding, declining or stable.

Another recent method is that by Wang and Whitlock (2003). The method can be used to estimate  $N_e$  "as usual", i.e. assuming that there is no gene flow into the population. In reality, however, this is often an invalid assumption, and gene flow may in fact have a significant effect on  $N_e$  estimates. Fortunately, the method can be used to take this into account. Pooled allelic data from populations that are likely to supply immigrants to the population in focus can be used to estimate immigration rate. Consequently, the method provides estimates of both  $N_e$  and migration rate.

#### 2.1.2.2 Other estimation methods

Alternatives to the temporal method include estimating  $N_e$  from linkage or rather gametic phase disequilibrium between two loci (Hill 1981; Waples 1991). Here, the principle is that genetic drift cause's gametic phase disequilibrium and the magnitude of such disequilibrium provides an estimate of genetic drift which again yields an estimate of  $N_e$ . It should be noted that this method can only be applied to a single cohort, and it provides an estimate of the *effective number of parents of the cohort* (and should correctly be denoted  $N_b$ ).

A related method consists of estimating  $N_e$  from heterozygote excess (e.g. Luikart and Cornuet, 1999). Again, the method estimates the number of parents of a single age class of individuals. The principle is that the lower  $N_e$  becomes, the more allelic frequencies will differ between the two sexes, simply as a result of stochasticity. When parents with different allelic frequencies reproduce, there will consequently be an excess of heterozygotes in the offspring, and  $N_e$  (or rather  $N_b$ ) can be estimated from this excess.

Finally, it should be noted that there are "non-temporal" methods available based on coalescent principles that can be used for joint estimation of various demographic parameters; e.g. MIGRATE (Beerli and Felsenstein 2001) for estimating migration rate and  $N_e$  and Beaumont's (1999) MSVAR, which can be used for estimating population declines and expansions, dating the decline/expansion and for estimating theta ( $4N_e\mu$ ), which can be transformed into  $N_e$  given

that  $\mu$  (mutation rate) is known. MIGRATE has so far been very little used in practise, but MSVAR has been used in a number of cases, though it has some weaknesses. It assumes a closed population, i.e. no gene flow, and consequently it is inappropriate in a number of cases including most marine and anadromous fishes. Furthermore, it assumes a strict stepwise mutation model, which is not completely valid for microsatellite loci.

### 2.1.2.3 Required criteria for the calculation of effective population size

As mentioned above, various assumptions are made in the calculation of effective population size, but the theoretical and mathematical basis for these is constantly being updated. The following conditions provide some practical guidelines as to the typical population suitable for effective population size estimates:

- a) a relatively “closed” population without large numbers of immigrants;
- b) a population for which a series of temporal samples are available e.g. otoliths or scale samples from archived collections compared with present day samples. An alternative source could be samples of fish collected at one point in time, comprising fish of different ages (each age therefore representing a different recruitment class);
- c) a population for which there is good demographic data e.g. knowledge of the survival and fecundity of each age class. It is possible to calculate effective population size from demographic data alone, but a combined approach whereby survey data can be used to target the most appropriate age groups for genetic analysis may also be helpful;
- d) a population within which there is no sub-structuring.

The general prerequisites for using the temporal method are that selection, migration and mutation are unimportant in relation to genetic drift and that there should be no substructure in the studied population. These assumptions are very important to be followed, because these factors might cause allele frequency changes in addition to genetic drift and especially for large populations, even very small changes might cause strong underestimation in the effective size estimation.

### 2.1.3 Potential sources of error when estimating $N_e$ from genetic data

#### 2.1.3.1 Technical errors such as mis-scoring, large allele dropout, and null-alleles

Microsatellite DNA PCR products sometimes show ‘stutter bands’ (thought to occur through slip-stranded mis-pairing) and the amplification of unspecific products that may erroneously be scored as alleles (Goossens *et al.* 1998 and references herein). Such problems seem especially prone in studies of marine fishes that commonly exhibit very high polymorphism (e.g. O’Connell and Wright 1997). These problems can be overcome by repeating PCR amplification and fragment visualisation multiple times for each sample. However, due to resource constraints, this procedure is commonly not carried out for more than smaller subsets (<10%) of samples. Consistent scoring of ‘false alleles’ will lead to spurious allele frequency estimates and inflate estimates of population heterozygosity. Conversely many rare small and large alleles can be systematically overlooked during scoring if the visualisation method used is set within an inadequate expected size range for PCR products. Whereas consistent scoring of false alleles may pose a problem in  $N_e$  estimation, failure to detect rare alleles should be of less significance unless scoring errors occur with a systematic bias, e.g. if temporal samples are scored using different visualisation techniques or different multiplexing set-ups. Most researchers try to minimise such scoring problems by preferably using microsatellite markers with tri- and tetranucleotide repeat units that are easier to score compared to dinucleotide loci.

Many  $N_e$  studies hinge on the analysis of historical samples such as otoliths and dried scales (e.g. Nielsen *et al.* 1999; Tessier and Bernatchez 1999; Koskinen *et al.* 2002; Hauser *et al.* 2002, Säisä *et al.* 2003). A potential source of scoring error that is especially pronounced in analyses of historical samples is large allele dropout. Historical samples normally contain highly degraded DNA, from which it is more difficult to amplify large compared to small alleles. Historical samples with low DNA content will also be especially prone to showing spurious peaks that may be scored as alleles (see above), leading to risk of elevating estimates of heterozygosity in historical samples compared to samples in which less degraded DNA is analysed. Large allele dropout may be especially common in marine fishes as their microsatellites are relatively large and with broad size ranges. Large allele dropout can be minimised by restricting analysis to short (<200 bp) microsatellite loci, and artificial peaks can be minimised by purifying and concentrating historical DNA prior to amplification.  $N_e$  estimation may be impaired if numbers and frequencies of detected alleles vary across temporal samples due to large allele dropout, mis-scoring, or a combination of both.

Null alleles are alleles that partly or fully fail to amplify during PCR and therefore are not scored. The most frequent cause of null alleles is thought to be point mutations within the primer site, but indels between primer site and repeat sequence, and indels within the repeat sequence leading to large differences in fragment size may also be common causes. Poor DNA quality may also lead to null alleles (see above). Null alleles can be partial if the allele is amplified when in homozygous condition (e.g. when its amplification is not impaired by that of another allele) but not when in heterozygous condition (when amplification is impaired by that of the other allele). Null alleles are reported in natural populations across most taxa, including many marine fishes (Jarne and Lagoda 1996), and there is general consensus

that they may contribute significantly to observed patterns of microsatellite variation. Null alleles may be detected through a signal of heterozygote deficiency and various statistical approaches are available for examining their presence (e.g. Brookfield 1996; Summers and Amos 1997; van Oosterhout *et al.* 2004).

#### **2.1.3.2 Low sample size and low allele frequencies**

Sampling too few individuals affects confidence levels on allele frequency estimates negatively and decreases the ‘signal-to-noise’-ratio on  $N_e$  estimates. This may be especially problematic in studies in marine fishes that generally exhibit large numbers of alleles. Using microsatellite data from Atlantic Cod, Ruzzante (1998) examined effects of sample sizes on estimates of genetic distance and population structure. He concluded that sample sizes of  $> 50$  and preferably around 100 are necessary for unbiased and precise estimates of genetic distances and structure. Numbers of alleles per locus were also found to affect bias, as loci with 30 alleles exhibited a relatively larger bias at low sample sizes than loci with ten alleles. The statistical properties of the temporal  $N_e$  method is generally unknown when loci with  $>12$  alleles are used (Waples 1989). Based on a simulation study, Turner *et al.* (2001) found that the accuracy of the temporal method decreases when sample sizes decrease and when the proportion of rare alleles per locus increases. Bias due to low allele frequencies also increases with increasing time intervals between temporal samples (due to low frequency alleles being constrained in how far downward they can drift). Sampling variance in allele number and rare alleles can lead to bias in estimation of  $N_e$ . Such effects can be reduced by Bayesian methods that use prior information incorporating all occurring alleles in all samples.

#### **2.1.3.3 Problems associated with obtaining temporal samples**

Many marine fishes have good dispersal abilities and the potential to move considerable distances. Most marine fishes of economic importance perform annual migrations between feeding, wintering and spawning areas that may be separated by hundreds of kilometers. A result of such migratory behaviour is local mixing between individuals originating from genetically differentiated populations, and a sample taken in a local site may contain individuals from multiple populations. Even samples taken at spawning sites may be a mix of residents and transients, as spawning times often vary among subpopulations (e.g. Atlantic herring, Atlantic cod). The high levels of heterozygosity and overall low genetic differentiation among marine populations (Ward *et al.* 1994) leads to low statistical power for detecting a Wahlund effect and whether sampling has taken place across genetically differentiated population components. Temporal  $N_e$  methods are based on the assumption that the same population can be sampled over time and that immigration from genetically differentiated population components is negligible (although the estimation approach by Wang and Whitlock (2003) enables relaxation this assumption). If the assumption of no migration cannot be fulfilled, the observed allele frequency variance may reflect population substructure instead of genetic drift and  $N_e$  estimates become unreliable. Temporal sampling of presumed reproductive units is fraught with difficulty in a marine environment. The best solution is to sample geographically and temporally stable spawning aggregations, using only ripe or spawning individuals as representatives of a reproductively coherent population component. If it is not feasible to sample spawners and if it is considered that there is no risk of sampling individuals originating from discrete populations, sampling outside spawning aggregations may be attempted. However,  $N_e$  estimates based on such sampling strategy should be treated with caution. In Atlantic cod, Knutsen *et al.* (in press) recently reported temporal variation in the genetic origin of juveniles present on spawning sites in the Skagerrak. Cryptic population substructure may occur even on spawning sites. In Atlantic herring, McPherson *et al.* (2003) e.g. found indications for genetically differentiated spawning waves.

#### **2.1.4 The $N_e/N$ ratio in marine fishes and shellfish**

Due to the difficulties of deriving realistic estimates of  $N_e$  in wild populations, the ratio  $N_e/N$  is of considerable importance to evolutionary biologists and conservation biologists. If it were possible to generate rules-of-thumb for predicting this ratio, then estimates of  $N_e$  could be generated from estimates of  $N$ , which are generally more available across taxa. Nunney (1993,1996) developed theoretical expectations for  $N_e$  under different mating systems, and concluded that for many species, single-generation  $N_e/N$  values would be in the order of 0.5 or greater. The prediction was tested empirically by examining the ratio for 100 species (Frankham 1995), and found the mean to be 0.53. Collectively, such findings suggest that  $N_e$  is approximately 1/3 to 1/2 of  $N$ .

Evidence to date indicates, however, that in many marine species (Table 2.1.4.1) the ratio  $N_e/N$  is considerably smaller. Studies have suggested that  $N_e$  can be considerably smaller (estimates range from  $10^2$  to  $10^6$  orders of magnitude less than census population sizes e.g. Turner *et al.* 2002; Hauser *et al.* 2002; Hutchinson *et al.* 2003).

Many of the species that show such low  $N_e/N$  ratios (e.g. oysters, anchovies, red drum, cod, New Zealand and Vermilion snapper) have Type III survivorship curves (i.e high fecundity and high mortality in early life stages). Hedgcock (1994) suggested that low  $N_e/N$  ratios in marine organisms could be explained by “sweepstake” survival of entire family groups during key life history stages. Under such a scenario, most families would produce no offspring that survive to adulthood because most larvae do not find suitable conditions for survival.

It is worth pointing out that most estimates of  $N_e$  to date are based on shifts in allele frequencies over the sampling period only; temporal method estimates are insensitive to bottlenecks that occurred prior or subsequent to sampling. Application of alternative methods (e.g. Turner *et al.* 2002), as estimated by a coalescence approach can detect histori-

cal bottleneck events. Such approaches take account of the differences in mutation rates and loss of alleles, as well as the equilibrium between genetic drift and mutation (Avisé 2000).

Table 2.1.4.1.  $N_e/N$  ratios for selected marine and freshwater species. Note that both the method of calculating  $N_e$  and the definition of  $N$  can affect the ratio. (**VF** Variance in gene frequencies, **LD** Linkage Disequilibrium, **T** Temporal Method, **MUT** mutation drift equilibrium).

Species	$N_e/N$	Method	Reference
Menhaden	<0.0025	MUT	Bowen and Avisé 1990
Black sea bass	0.005	MUT	Bowen and Avisé 1990
Pacific oyster	<0.000001	VF	Hedgecock <i>et al.</i> 1992
Sea bass	0.27 – 0.40	LD	Bartley <i>et al.</i> 1992
Chinook salmon	0.013 – 0.043	LD	Bartley <i>et al.</i> 1992
Steelhead trout	0.73	T	Ardren and Kapuscinski 2003
New Zealand snapper	0.00001	Various methods	Hauser <i>et al.</i> 2002
Red drum	0.004	T	Turner <i>et al.</i> 1999
Red drum	0.001	T	Turner <i>et al.</i> 2002
Vermilion snapper	0.0015–0.0025	LD	Bagley <i>et al.</i> 1999
Northern pike	0.03 – 0.14	T	Miller and Kapuscinski 1997
Atlantic cod	0.00004	T	Hutchinson <i>et al.</i> 2003
Chinook salmon	0.02 – 0.56	Various methods	Shrimpton and Heath 2003

### 2.1.5 Factors affecting the $N_e/N$ ratio

There are many potential factors that may contribute to the small  $N_e/N$  ratio observed in marine fishes. Demographic factors such as **population fluctuations** are likely to be crucially important (Vucetich *et al.*, 1997), yet many of the early modelling studies of  $N_e$  assumed constant population size (Nunney, 1995). Many marine fish populations experience cyclical changes in population size, referred to as “boom and bust” cycles (Blaxter and Hunter, 1982). These may be caused by great natural variability in recruitment from year to year, often associated with environmental factors such as climate change or the availability of prey (Beaugrand *et al.*, 2003). Additional factors such as fluctuating hydrographic conditions or productivity may also affect recruitment (Grant and Bowen, 1998).

Even if population sizes remain constant across several years, **high variability in reproductive success** (arising from both variance in **family** reproductive success, and variance in **lifetime** reproductive success) among individuals may limit effective population sizes due to the fact that a minority of individuals may contribute to the gene pool at any one time. Variability in recruitment among families has been demonstrated for some finfish species, identified by spatially and temporally distinct groups of larvae that represent a biased sample of the genotypes present in the previous generation. Ruzzante *et al.* (1996) analysed an aggregation of cod larvae taken from within a gyral water mass sampled repeatedly over three weeks. Microsatellite analysis showed strong evidence for departures from Hardy Weinberg expectations for the larval aggregation as a whole and for a subset of the larvae found within a single water mass, suggesting the existence of several heterogeneous groups. Analysis of larvae grouped by age-at-length (potentially belonging to the same cohort) failed to detect such strong patterns. The authors suggested that the larval aggregation as a whole was composed of individuals from several distinct spawning events, among which there were marked differences in allele frequencies. The larvae forming the cohort could have originated from a single spawning event, although additional analyses on a sub-set of the same samples failed to detect any family structures (Herbinger *et al.*, 1997). Such studies highlight that spatially or temporally distinct cohorts of cod larvae can exist and be influenced by changing conditions in the local environmental that may result in large differences in survival (match-mismatch hypothesis; Cushing, 1972). Additionally, studies have been carried out that directly examine variation in the abundance of discrete cohorts in white sea bream (Planes and Lenfant 2002; Lenfant and Planes 2002), indicating considerable variance in reproductive success.

Variance in both **lifetime** and **family reproductive success** has been implicated in the low effective population size observed in the New Zealand snapper (Hauser *et al.*, 2003). Snapper are long-lived and have strong weight-dependant fecundity such that old fish are likely to contribute disproportionately to recruitment. Older and larger fish also produce larger eggs, which may improve the survival and growth of their offspring. In addition, the population studied was at the southern edge of the species distribution and cold conditions during El Niño years often result in complete recruitment failure.

**Historical demographic events** such as population bottlenecks or founder events will affect  $N_e$  in a similar way to cyclical fluctuations in population size. Whilst bottlenecks associated with changes in a species range over evolutionary time can be studied using long-term effective population size, anthropogenically induced bottlenecks may affect  $N_e$  in the shorter term. Shrimpton and Heath (2003) compared census and effective population size in five populations of Chinook salmon in Canada over 20 years. The populations have been affected by both small numbers of initial founders and subsequent population bottlenecks caused by large-scale habitat perturbations associated with forest and construction activities and sedimentation. Results showed that whilst observed population sizes appeared to increase, estimates

of  $N_e$  decreased over the period. Spawning habitat area was found to be correlated with  $N_e$  suggesting that anthropogenic activities have reduced the carrying capacity of the tributaries and contributed to the reproductive failure in a subset of the populations.

**Behavioural or life history factors** can also alter effective population size. A comparative study on populations of Pacific sardine (*Sardinops sagax*) and northern anchovy (*Engraulis mordax*) in the California Current System has demonstrated that whilst these species exhibit similar census population sizes, moderate differences in life history have a strong effect on  $N_e$  (Gaggiotti and Vetter, 1999). The differences were largely related to the fact that the reproductive value of northern anchovy populations was much greater than that of the Pacific sardine, particularly in the early age classes. The two species also had profoundly different levels of genetic diversity, with the northern anchovy displaying levels of heterozygosity seven times higher than the sardine (Hedgecock *et al.*, 1989). Such differences may in part be related to life history, as the Pacific sardine has a long life span, late age at maturation and low offspring survival rate relative to the northern anchovy, but may also be related to the heavy exploitation of Pacific sardine between 1945 and 1960. Any effect that reduces the overall effective size of a population is likely to mean that fishing has an enhanced impact on the underlying levels of genetic diversity. Such studies simply highlight the importance of understanding effective population size and the use of applying genetic techniques to fishery management.

**Mating patterns** can have important implications for the demographic and genetic structure of marine fish populations (Rowe and Hutchings, 2003). In some taxa, e.g. birds and mammals, non-random mating is known to lead to reduced effective population size and spatially discrete genetic cohorts, which may be sired by relatively few potential breeders. Non-random mating may reduce  $N_e$  if, for example, there is bias in the proportion of males that are reproductively successful. Atlantic cod are broadcast spawners, a feature that has traditionally been thought to limit the degree of mate choice and restrict the capacity for complex behavioural patterns (Berglund 1997). However, recent studies have shown that cod reproductive behaviour shares some of the characteristics of lekking systems (Nordeide and Folstad, 2000), such that complex behaviours may indeed determine mating success of males and females within aggregations. Brawn (1961) showed that cod reproduction involved males courting females, and that gametes are released as the male and female swim together in a “ventral mount”. Such behaviour can be preceded by acoustic communications by males (Brawn, 1961), circling bouts by males around females positioned on the bottom, and male to male antagonistic behaviour, which has been associated with a size based dominance hierarchy (Hutchings *et al.* 1999). All such factors could facilitate mate choice and a mechanism for biased reproductive potential between variable quality mates.

Very few genetic studies exist of mating behaviour in commercial finfish species although a recent example highlights the potential of this effect. Male reproductive success of cod was analysed in a sample of 50 breeding adults kept in experimental sea enclosures across a spawning season in Norway. Assignment of offspring to parents was possible using four microsatellite loci and showed that the average number of fathers contributing to a single spawning event was 2.12 (SE = 0.08) (Bekkevold *et al.* 2002). However, genetic analysis indicated that paternity was highly skewed among males with larger males siring a greater proportion of offspring. Male reproductive success was also dependant on the size difference between a female and male, and supported previous observations of lekking structures in cod. While this study was conducted on an experimental population, it suggests that mating patterns in natural populations of cod are complex. Moreover, biased reproductive success of males may contribute towards the presence of greater than expected numbers of related individuals. In addition, the relationship between male /female size and reproductive success may also have implications for cod populations experiencing fishery induced changes in size maturation and growth rates (Beacham 1983; Law 2000).

#### **2.1.6 Identifying alternative methods for evaluating the validity of $N_e$ estimates: genetic, demographic and life-history data**

$N_e$  from temporal variance estimates can be validated using other genetic data as well as demographic and life-history data and by simulation. Simulated genetic data which incorporate published rates of technical error and known levels of biological sources of variance (for instance cohort effects and microgeographical genetic differentiation, e.g. in Hutchinson *et al.* 2001; Knutsen *et al.* 2003; Nielsen *et al.* 2003; 2004) can be modelled and used to explore the sensitivity of temporal methods for estimating  $N_e$ .

Another approach is to assess the internal consistency of the data sets and results. For example, the levels of genetic variation (for instance number of microsatellite alleles) should be compatible with the  $N_e$  estimates based on temporal variance in allele frequencies. This is explained further: If the hypothesis is that a low  $N_e$  or  $N_e/N$  ratio has arisen through natural causes (see 2.1.5 above), the population in question can be assumed to be at mutation drift (see Bowen and Avise 1990) equilibrium. Using the simple infinite allele model (Kimura and Crow 1964) as the mutation model (and this may not be the most appropriate for microsatellite loci), it requires extreme mutation rates ( $10^{-2}$ ) to have more than 10 alleles at a microsatellite (or any other locus) at mutation/drift equilibrium for  $N_e$  of less than 500. Therefore, if a population has a large number of alleles and is assumed to be in equilibrium, then the theoretical models are incompatible with very low estimates of  $N_e$ .

Levels of variation at microsatellite loci in marine fish are generally higher than 10 alleles per locus (e.g. O’Connell and Wright 1997). For example (Nielsen *et al.* 2003) found seven of nine microsatellite loci to have more than 15 alleles with a maximum of 50 alleles at one locus in a sample of 79 cod from the North Sea. However, a study of turbot from the same area (Nielsen *et al.* 2004) revealed only one of eight loci with more than 10 alleles. For cod other genetic data are also available for comparison. Pogson and Fevolden (2003) found a total of 127 alleles at the nu-

clear *Pan I* locus. Along the same lines a number of studies by Árnason *et al.* (1998) have revealed more than 34 mtDNA haplotypes at cytochrome b in cod. More data is obviously needed in order to evaluate general patterns of variability in marine fishes, however, based on present data primarily from cod, effective population sizes ranging in the 100's seem to be incompatible with observed levels of variability in general.

However, most wild populations are probably not in mutation-drift equilibrium, possibly accounting for such disparity in allelic variability. If this is the case, the above mentioned method (i.e. assessing internal consistency of the data) cannot be employed. Instead coalescent methods have to be engaged. Coalescent theory can be used to determine the number of ancestral lineages at some point backwards in time given the number of alleles observed in a sample today. The coalescent approach can be used to evaluate the maximum number of alleles in a population given different scenarios. For example a fishery-introduced bottleneck around a hundred years ago could lead to low effective population size today. Poulsen *et al.* (unpublished) investigated a scenario where a population bottleneck 25 generations ago lead to an effective population size of only 100 which coalesced to 15 lineages at the beginning of the bottleneck. In other words, this scenario suggests a maximum of 15 alleles in the population today.

The validity of genetically based  $N_e$  estimates can also be assessed in comparisons with estimates from demographic data. Demographic  $N_e$  estimates are based on parameters that are theoretically known to affect  $N_e$ , such as fluctuating population size, biased sex ratio, mating system, selection and variance in family size (Wright 1938). Several estimation methods have been formalised and applied in natural populations recently (e.g. Nunney 1996; Scribner and Chesson 1996). Whereas many of the parameters used in demographic  $N_e$  studies are at best difficult to estimate in natural populations in a marine environment, the approach can be used to evaluate the parameter space that must be fulfilled in order to explain estimates obtained from genetic methods. More specifically, if genetic  $N_e$  estimates in marine fishes generally suggest very low  $N_e/N$  ratios (see above) it is pertinent to ask what demographic or life-history traits may be responsible for such low ratios. Likewise, is it possible to develop models for the genetic processes that must be fulfilled for genetic  $N_e$  estimates to 'make sense' biologically? For instance, if  $N_e/N$  ratios are in the order of  $10^{-5}$ , then - *a priori* - this requires a very high proportion (several percent) of eggs spawned over the entire lifespan of a reproducing female to survive to reproduction.

### 2.1.7 Significance and implications

Many factors contribute to the uncertainty in deriving estimates of stock recruitment relationships (Myers and Barrowman 1996; Myers 1997). In the light of theory and empirical estimates it is worth while considering within the fisheries context the extent to which disparities in effective and census population sizes may contribute. Moreover, the maintenance of a sufficiently high  $N_e$  is a main priority in conservation biology order to maintain levels of genetic variation to maximise evolutionary potential.

In addition to the implications of the low  $N_e/N$  ratios on estimates of stock-recruitment relationships, at least two recent molecular genetic studies on marine fishes (New Zealand snapper, *Pagrus auratus*; Hauser *et al.* 2002, and North Sea cod, *Gadus morhua*, Hutchinson *et al.* 2003) have indicated that population declines linked to over fishing have been associated with rapid genetic change, a reduction in genetic diversity, and possible local population displacement. In the case of *P. auratus*, for example, strong evidence for genetic drift and loss of rare alleles was detected during a recent marked population decline, though it was estimated that the minimum population size never fell below 3 million individuals. The small  $N_e/N$  ratio ( $10^{-5}$ ) indicates that drift and loss of diversity may occur in populations with high census counts. Although genetic diversity was assessed using molecular markers assumed to have no selective significance ("neutral"), and although the relationship between molecular and adaptive variation is complex (Carvalho *et al.* 2003), such rapid genetic change could be associated with a reduced evolutionary potential. Such assertions have particular significance when considering conservation of genetic resources. A general rule-of-thumb in conservation biology is that the minimum  $N_e$  for short-term conservation is 50 (1), and the minimum  $N_e$  for long-term adaptability is 500 (2). If we substitute an  $N_e/N$  ratio of  $10^{-5}$  taken from the snapper example above, the minimum census sizes to maintain variability would be  $5 \times 10^6$  (1) and  $50 \times 10^6$  (2) respectively. Such data not only indicate that even very large marine fish populations may be vulnerable to overexploitation and environmentally-induced recruitment failure, but also importantly that fishery models need to take account of genetic estimates of population size if sustainable forecasts are to be achieved.

### 2.1.8 Recommendations

- 1) More studies are needed among and within species where population genetic data, demography and life-history traits are well documented to determine the generality of the  $N_e/N$  patterns observed so far. Such studies should include considerations such as temporal stability as well as population and species specific heterogeneity.
- 2) In order to assess the relative contribution of historical and contemporary processes on  $N_e/N$  ratios, additional studies that assess long term variance in  $N_e$  estimates (e.g. using coalescence theory) are required.
- 3) Prior to undertaking analysis it is crucial to design a sampling programme in accordance with the underlying assumptions necessary for generating precise  $N_e$  estimates (e.g. that the population is relatively closed and without significant substructure).

- 4) In addition to optimal sampling design, it is imperative to exercise sound quality control of genetic data (e.g. genotyping consistency) prior to statistical analysis.
- 5) It is recommended that estimation methods are validated through simulation and theoretical studies, in order to quantify the contribution of genetic marker variability and technical artefacts, such as null alleles, mis-scoring, large allele drop out, on  $N_e$  estimates, and to identify the limitations of the available methods.
- 6)  $N_e$  and  $N_e/N$  estimates and their long term trends should be examined over successive temporal sampling dates and across generations to test signal-to-noise ratios.
- 7) To address the uncertainty inherent in fulfilling the assumptions needed for precise  $N_e$  estimation it is recommended that independent methods are employed simultaneously to test for concordance.
- 8) Significant disparities between  $N_e$  and  $N$  should be considered in stock assessment models to reduce uncertainty in recruitment relationships.

### 2.1.9 References

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## 2.2 Evaluate the management recommendations for Atlantic salmon, developed by the SALGEN EU project (ToR b).

SALGEN ([www.salgen.marlab.ac.uk](http://www.salgen.marlab.ac.uk)) is a project set up to review genetic studies on Atlantic salmon and develop management recommendations for the species. It was expected that the project topic reviews and management recommendations would be published in book form in early 2004. However the project has encountered some production delays and will not report until late summer 2004. Unfortunately as a consequence WGAGFM have been unable to undertake the envisaged review at this time. It is recommended that the review of SALGEN project outputs be removed from the WGAGFM terms of reference. It would be appropriate for WGAGFM to provide a critique on the finished publication.

## 2.3 Consider conservation genetics aspects required for conservation targets for eels (ToR c).

This section is based on a position paper prepared by J. Trautner, F. Volckaert, R. Hanel and P. McGinnity and adopted by the WGAGFM in Hamburg in 2004.

### 2.3.1 Introduction

The European eel (*Anguilla anguilla* L.; Anguillidae; Teleostei) is described as a catadromous species throughout the literature. It spends a major part of its life in freshwater or estuaries; but larval (leptocephali) and postlarval (glass eel) stages as well as the mature adults (silver eels) occur in the open ocean. Males start migrating at the age of 7–8 while females start at around 11 years of age (Tesch 1977). Based on the observed geographical distribution of newly hatched larvae, the European eel is believed to spawn in the Sargasso Sea (Western Atlantic) although spawning eels have never been caught in this area. The spawning season lasts from late winter to early summer. After spawning the adult eels do not return into freshwater or estuaries and seem to die (semelparous reproduction). The larvae drift eastwards to the continental shelf of Europe but also active migration seems to be involved (Lecomte-Finiger 1994).

During their journey the larvae undergo metamorphosis through a leptocephalus larva to the glass eel stage, when approaching the continental margin. As they approach the coast pigmentation starts and transforms the glass eel into the

yellow eel stage. A currently unknown portion of the eels is ascending European and North –African river systems, while many eels seem to remain in the estuaries and the marine environment, close to the coasts and therefore have a complete marine life cycle. This has been proven by otolith microchemical analysis (Tsukamoto *et al.* 1998a). The European eel remains one of the most mysterious fish species heavily exploited by humans.

### 2.3.2 Current Status of the European Eel Stock

The total European eel stock has been steadily declining since the 1940s with a total catch of 40 000 tonnes a year with a peak in the 1960s - coinciding with a peak in re-stocking - reaching an all time low in the 1990s of less than 20 000 tonnes a year (Dekker 2003a). Since the early 1980s, recruitment has steadily declined (Moriarty 1986) as observed at most monitoring sites (Moriarty 1986, 1990, 1996; Dekker 2000). It is nowadays down to an alarming average of 1 % of former values (Dekker *et al.* 2003b; ICES/EIFAC WG EEL 2004). The major problem in estimating the stock size of European eel is the lack of monitoring programs; consequently all estimates are more or less based on catch data. Nevertheless, the decline in stock and recruitment is obvious - merely the magnitude of the decline is contentious. Different reasons are believed to be responsible for the dramatic decline.

In 1998 the European Commission requested ICES to advise on the management of European eel:

*“There is an increasing concern about the situation for the European eel stock and its future development. ICES is therefore requested, to provide information about the status of eel stock(s) and on any possible management actions, and to identify gaps in knowledge about eel in order to secure a sustainable development of the eel fisheries within the European Union.”*

ICES delivered a comprehensive report and stated that currently the stock is outside safe biological limits.

The European Commission reacted to the decline with the “Development of a Community Action Plan for the management of European Eel” (Commission of the European Communities 2003) which is currently developed. It turned out, that the major problem in developing such a plan is the lack of reliable data for abundances and distribution of the certain life stages. Most estimates are imprecise, necessitating the establishment of a comprehensive monitoring plan as a matter of urgency.

### 2.3.3 Threats to the European Eel

#### 2.3.3.1 Fishing Pressure

The fishing pressure on eel refers to the last three stages. The glass eel fishery takes place preferably at river mouths and in coastal regions. Glass eels are used for human consumption, mainly as traditional food in Spain and Portugal. Large quantities are exported to Asia where the eels are used as “fingerlings” in farms for eel production. Part of the catch is stocked as fingerlings in European farms and used for the restocking of natural habitats (within and between basins). There are no numbers on the transfer of glass eels between basins. The yearly catch of glass eel has dramatically declined in the last few years leading to the estimation of a 99% reduction in recruitment (Dekker 2003b). The larger sized yellow eel and silver eel are caught mostly in lowland rivers for sale on the fresh market. As mentioned above, there is insufficient information on actual catch data and stock size to demonstrate extensive fishing as the main reason for the decline.

#### 2.3.3.2 Parasites

The nematode *Anguillicola crassus* is a serious threat to growing and adult eels living in fresh water. The nematode is found in the gas bladder of yellow and silver eels and affects its buoyancy function (Figure 2.3.3.2.1). It was accidentally introduced into Europe in the early 1980s, most likely through imports of Japanese eels to Germany (Koops and Hartmann 1989; K  ie 1991) and a copepod is believed to be the vector (F. Volckert pers. com.). The infection with *A. crassus* might be one reason for a lower return rate of silver eels to the Sargasso Sea due to a negative effect on the energy budget as active swimming is required to avoid sinking (Kirk 2003). This is currently under investigation in an EU sponsored research programme entitled “Estimation of the reproductive capacity of European Eel” (EELREP) (2001–2004). The monogenean trematode worm *Gyrodactylus anguillar* commonly infects the gills and causes much concern in aquaculture, though the impact in nature is not known.



Figure 2.3.3.2.1. The introduced parasitic nematode *Anguillicola crassus* is found in the swim bladder of 80 to 100% of silver eels.

#### 2.3.3.3 Viral and Bacterial Infections

The microbiology of pathogens infecting eels is understudied. Viral infections regulate natural populations and cause a lot of problems in aquaculture. The newly introduced virus EVEX from the Far East, is a cause for concern. It is currently under investigation in an EU sponsored research programme entitled “Estimation of the reproductive capacity of European Eel” (EELREP). The bacteria *Aeromonas salmonicida* (red mouth disease) and *Vibrio anguillarum* causes mass mortalities in aquaculture.

#### 2.3.3.4 Oceanic and Climatic Changes

Not only biological and human factors are considered possible reasons for the decline. The connection between the recruitment decline in European eel and a decadal scale change in the oceanic circulation (North Atlantic Oscillation – NAO) points towards those fluctuations as a possible cause of the decline. The parallel decline of the recruitment of the American eel in some of its distribution areas, and the correlation between the recruitment and the North Atlantic Oscillation anomaly supports this model (for sprat see MacKenzie and Koster, 2004).

#### 2.3.3.5 Human Activities (other than fishing)

River fragmentation by dams and hydro-electric power plants is considered a huge problem to migrating silver eels. A case study at German power plants revealed that 5–25% of the returning silver eels get caught and die in the turbines of every single facility (Larnier and Darfiguelongue 1989). As many of these facilities are to be found throughout the length of a river system, the death rate adds up dramatically and exceeds the overall fishing mortality. But as it is thought that a portion of the European eel never enters freshwater habitats, river fragmentation might not be a major reason for the decline.

Pollution by organic chemicals and heavy metals also seems to be an important factor partially explaining the dramatic decline in recruitment. In some regions as e.g. in The Netherlands and Flanders the eel is so heavily polluted that human consumption is prohibited (Goemans *et al.* 2003). Several of the toxic substances like organochlorines, pharmaceuticals and endocrine disruptors have a negative effect on fecundity.

#### 2.3.4 The Genetic Structure of the European Eel

Over a very long period of time the European eel was considered a panmictic species with no significant population genetic structure evolving. Evidence was based on predicted oceanic drift randomly dispersing the larvae to the coasts of Europe and into the Mediterranean, and the more or less simultaneous mating of European eels in the same area, the Sargasso Sea.

The development of population genetic structure necessitates spatial or temporal separation of certain groups occurs. Hence if structuring is the case, at least one of the above assumptions must be rejected: either the eels do not spawn simultaneously or/and there is more than one place where mating occurs and homing behaviour for these places must exist. Evolution of a homing behaviour for certain rivers or coastal regions should not be possible without concurrent spatially separated spawning areas. Until now there is no hard evidence for any of these scenarios from observa-

tions of the abundances of specific life stages, such as the spatial and temporal distribution of larvae. However, there is also no evidence against it.

As classical research approaches did not lead to a consensus yet, genetic methods should provide hopeful means to address the problem. In early studies on the genetic structure of European eel based on allozymes, genetic differentiation between samples from different locations was suspected (Drilhon *et al.* 1967; Pantelouris *et al.* 1971) but the conclusions were disputed on methodological reasons (Koehn 1972). Later studies using the same methodology (Comparini and Rodinò 1980; Yahyaoui *et al.* 1983) did not reveal geographical differences. On the contrary temporal stability of genetic differences between geographic regions was shown using the same kind of marker (Williams *et al.* 1973; Koehn and Williams 1978), but the differences were interpreted as selection.

Results from DNA sequence data of the mitochondrial D-loop region, served as a basis for the conclusion that Mediterranean and North-eastern Atlantic eel belong to a single population (Lintas *et al.* 1998; Daemen *et al.* 2001). In the former study the sequence differences ranged from 0% to 6.33% with an average value - SD of 3.01% - 1.18% between the 51 haplotypes observed. No correlation between geographic and genetic distance, thereby supporting the panmictic nature of European eel. In the latter study cytochrome *b* sequences (392 bp) were less diverse (17 haplotypes for 107 fish from Iceland, Ireland, Great Britain, Sweden, Italy and Morocco) and differed up to 4.8%. An increase in haplotype number was observed with latitude; the  $F_{ST}$  amounted to 0.014.

Recent population genetic studies using the more powerful technique of DNA fingerprinting with microsatellite markers aimed to solve the population structure in eel. Daemen *et al.* (2001) used 5 markers on 6 samples of silver and yellow eel (253 individuals) to detect a very weak but significant differentiation ( $F_{ST} = 0.004$ ). Temporal replications were only present for the samples from Ireland and revealed a pair-wise non significant  $F_{ST}$  value of 0.023 between these two samples. The only significant pair-wise differences observed were those given by the comparisons of the samples from Morocco to the samples from all other locations. Hence a significant isolation by distance pattern was not observed. According to the authors, the results should be interpreted with caution due to the limitation of the data (see further). A second study (Wirth and Bernatchez 2001) where 7 microsatellite loci were investigated revealed that the European eel shows a weak pattern of isolation by distance; as a result, panmixia was refuted by the authors. A total of 13 samples (611 individuals) ranging from Iceland through Norway, UK, France, and Portugal down to Mediterranean locations in Algeria, Italy and France were analyzed. The samples were all taken in spring or autumn 1999 as glass eel and in some locations as yellow eel. The observed genetic differentiation was low with an  $F_{ST}$  of 0.0017 but significant ( $p=0.0014$ ) and in the same range as  $F_{ST}$ s for other marine species (deWoody and Avise 2000). Pair-wise comparisons between Northern and Mediterranean samples revealed weak but significant  $F_{ST}$  values from 0.003 to 0.005. A possible sampling error as cause for the observed differentiation was argued against by the observed significant correlation between genetic and costal distance with a correlation factor  $r=0.460$ . The authors support a model explaining the observed structure through a temporal delay between the arrivals of adult eels from different latitudes at a common breeding site. The observed spawning season from late winter to early summer (Schmidt 1925) might reflect the successive arrival of waves of the reproductive migration.

The main weakness of these studies is (1) the lack of samples of the same age (there are glass eel, yellow and silver eels involved), and (2) the lack of samples from different years to show temporal stability of the observed structure. The argument that the observed structure is correlated with geographical distance is not very robust as this may have happened by chance. Effects like year class strength can not be excluded, as not always the same life stages or year classes (cohorts) were sampled.

In a later study where the same microsatellite loci were used to analyze population structuring in the American eel (*Anguilla rostrata*) no isolation by distance was observed and hence no evidence against panmixia was described. The observed  $F_{ST}=0.0022$ ,  $p<0.01$ ) was described as very low and possibly caused by sampling error alone (Wirth and Bernatchez 2003).

Isolation by distance was also one of the possible explanations in a study of the genetic variability of European eel using 15 allozyme loci (Maes and Volckaert 2002). Seven loci were polymorphic, the observed genetic differentiation was once more low ( $F_{ST}=0.002$ ) and explained by the high dispersal capability of the species. Two loci were responsible for the geographical cline. Three distinct groups were identified: Northern Europe, Western Europe and the Mediterranean Sea. Again, the mixing of age classes and the lack of temporal replicates can be considered weak points.

Summarizing the available knowledge here leads to a yet undefined genetic structure of European eel. Mitochondrial haplotypes, allozymes and microsatellites show high levels of genetic diversity, pointing to the long evolutionary history of European eel and the large population sizes. Genetic differences between geographic regions are present but have not yet been confirmed to be stable in time. Isolation by distance of several spawning populations is only supported by a two studies lacking temporal replication.

The spatio-temporal genetic variation in European eel is described in a recent publication (Dannewitz 2003). The study was intended to fill knowledge gaps on the temporal stability of genetic structure. He sampled and analyzed many more geographic locations with considerable temporal replication. In sharp contrast to the above studies and interpretations, there was no significant inter-location genetic heterogeneity and hence no isolation by distance found in this study. Instead, hierarchical analysis revealed that genetic variation among temporal samples (glass eel cohorts of 1994, 2000 and 2001) within sites clearly exceeded the geographical component. The authors hypothesize that temporal genetic variation plays a significant role in explaining the spatial structure reported earlier for this species. Hence, a factor overlooked in many studies, namely time, might play an important role in structuring European eel.

Population dynamical estimates of European eel, such as effective population size, among cohort variation and relatedness among arrival waves of new recruits are under evaluation. Marine populations are known to not have such huge effective population sizes as their census sizes might suggest (ICES-WGAGFM ToRa 2004 Effective population size). The sharp drop in census population size might have an impact on genetic diversity, but sound reference data compromise any conclusion. In addition the selective forces operating during the freshwater life stages are under investigation. At the moment such information is not yet available.

Another aspect which requires attention is the status of Icelandic eels. There is an overlap in the range of American and European eel in Iceland, which might result in hybridisation between both species. Phylogenetically *Anguilla rostrata* and *A. anguilla* are related but distinct species (Aoyama *et al.* 2001). Based on morphology (the number of vertebrae), allozyme and mitochondrial DNA variation, Avise *et al.* (1990) documented some level of introgression. Mank and Avise (2003) could not confirm this from microsatellite genotypes. Pending proof, the suspicion remains that introgression is ongoing in the Icelandic eels.

Finally, the lack of genetic differentiation in recent studies does not exclude the absence of adaptive variation (phenotype). No such study has been performed on European eel, but evidence from other fish shows that despite marked phenotypic plasticity, rapid genetic change can occur (for cod: Olsen *et al.* 2004). In invertebrates the significance of the phenotype (as measured with the  $Q_{ST}$ ) in discriminating between populations reaches values of ten or more times larger than the genetic differentiation (as measured with  $F_{ST}$ ) (Cousyn *et al.* 2001).

In summary, Icelandic populations of European eel tend to differentiate from European populations. The latter show a weak but significant geographical differentiation, which is not stable in time. Adapted sampling strategies might clarify matters in the near future.

### 2.3.5 Goals for the Conservation of Genetic Diversity in European Eel

In August 2003 the eel research community prepared the Québec Declaration of Concern (Dekker *et al.* 2003) given the immediacy with which eel populations are declining worldwide. No specific reference is made to the genetic implications of such decline. Therefore the following seven points should be considered as complimentary to the Québec Declaration of concern.

### 2.3.6 Recommendations

- 1) The genetic structure of natural populations can be best undertaken by identifying and sampling discrete reproductive aggregations. This is difficult because the European eel spawns in an area that is not well delineated or sufficiently accessible to fishing. Since Schmidt identified concentrations of eel *leptocephali* around the Sargasso Sea in the 1920s there has been little progress in locating eel spawning areas. However it is likely that recent advances in physical oceanography offer a reasonable opportunity of overcoming this deficit in the near future. In addition, tagging and tracking of fish has progressed such that monitoring from feeding to spawning ground is feasible.

*It is recommended that work is undertaken to delineate the spawning grounds.*

- 2) There are difficulties in effectively sampling the putative populations on the continental shelf because of the confounding effect of overlapping generations in adult migrants. This makes that the population genetic structure of the species is as yet not fully elucidated. Icelandic stocks seem different from North-eastern Atlantic stocks. For the latter group there is sufficient recent evidence available to suggest that small but significant levels of genetic structuring exist and that this diversity should be protected.

*Eel fisheries management should be conscious of subtle genetic structure and follow the precautionary principle.*

- 3) From evolutionary theory we know that absence of measured genotypic differentiation or small level of genetic differentiation at the molecular level does not preclude significant quantitative or adaptive differentiation. Levels of quantitative differentiation can be determined using reciprocal “common garden” experiments, where the phenotypes of mutual strain transfers between environments can be compared. Such experiments have not yet been undertaken for the eel; they should be undertaken in the near future. In the absence of experimental data it should not be assumed that such differences do not exist.

*It is recommended here that the precautionary principle be adopted to protect as of yet unresolved genetic variability, and as a consequence the transfer of glass eels between basins should be avoided.*

- 4) Existing census data indicates that the eel is in serious decline over most of its range. It is essential that the spawning stock/stocks of eel be maintained at sufficiently large levels to ensure that effective population sizes ( $N_e$ ) as well as absolute population sizes are optimized beyond safe limits. In order to protect genetic diversity it is important that eels from all regions throughout the range contribute proportionately.

*We recommend that sufficient adult recruitment is ensured to maintain a large and spatially representative silver eel population.*

- 5) As a consequence, efforts should be made to accurately estimate the total and proportional return rates of marine and freshwater matured silver eels to the spawning grounds. Good use should be made of the existing stock assessment indices as a surrogate for actual recruitment.

*It is recommended that support be given to current EIFAC/ICES initiatives to collect better spawning stock size data.*

- 6) One of the few ways of monitoring the genetic health of stock size is by examining allele frequencies at a large number of highly variable microsatellite loci over time (a loss of a substantial number of alleles would be indicative of a reduction in Ne). Many fisheries institutes and museums have good historical otolith collections, which could be used for this type of temporal genetic analysis.

*It is recommended that an inventory of European eel otolith archives be made. This database should include information regarding the condition of the samples in the archive and methods used to preserve the samples.*

- 7) Also the sex ratio of the eel is strongly affected by the environment (temperature, stocking density and chemicals). Shifts in sex ratio occur due to anthropogenic factors (e.g. farmed and restocked eels have a large proportion of males; endocrine disruptors affect the reproductive system). Skewed sex ratios have the potential to reduce effective population size (Ne).

*Information on the sex composition of migrating silver eels should be routinely collected in ongoing and future monitoring programmes.*

### 2.3.7 References

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## 2.4 Evaluate the use of reaction norms to evaluate the genetic impact of selective fishing (ToR d).

This section is based on a position paper prepared by U. Dieckmann, B. Ernande, M. Heino and P. Boudry and adopted by theWGAGFM in Hamburg in 2004.

### 2.4.1 Introduction

Today, fishing is the dominant source of mortality in most commercially exploited fish stocks. According to the United Nations' Food and Agricultural Organization, world capture fisheries have reached a ceiling, with three stocks out of four being maximally exploited or overexploited. Since all fish species were genetically adapted to the environmental conditions experienced prior to intensive exploitation, the current, drastically altered conditions cannot possibly leave their life-history patterns unaffected. In other words, fishing not only decreases the abundance of fish in exploited fish stocks, but also changes their genetic composition. This evolutionary dimension of fisheries has been overlooked or downplayed for decades, so that fisheries scientists and managers are just now awakening to the formidable risks posed by further unmanaged fisheries-induced evolution.

The awakening of attention towards questions of fisheries-induced evolution appears to have been facilitated by two independent developments. First, over the past decade the formerly narrow focus in fisheries management has been widened, from the traditional goal of avoiding overfishing and securing maximum yield, to an ecosystem perspective recognizing a much wider range of potential threats to marine biodiversity and its constituents. Consequently, in compliance with the FAO Code of Conduct for Responsible Fisheries, most major fishing nations in the North Atlantic have recently agreed to respect a precautionary approach to “conservation, management and exploitation of living aquatic resources in order to protect them and preserve the aquatic environment, taking account of the best scientific evidence available”.

As a second factor, a suite of scientific advances have suggested that fisheries-induced evolution is ubiquitous and that, if unmanaged, such processes may pose serious threats to exploited stocks and to their value as resources for humankind:

- There is growing recognition that fishing causes severe changes in the demographic properties of exploited stocks. This applies, in particular, to maturation: in most stocks, fish mature earlier today than they used to only a few decades ago.
- At the same time it has been shown that earlier maturation may have adverse implications for the reproductive potential of fish stocks, not only because large females produce more offspring per unit of body weight than smaller ones, but also because the size of females and the quality of their offspring tend to be positively correlated.
- Furthermore, thanks to recently developed improved statistical methods, it is becoming increasingly clear that most of the documented changes in maturation are indeed evolutionary responses, and not mere effects of phenotypic plasticity.
- Corroborating theoretical expectations, it has recently been demonstrated experimentally that size-selective fishing can cause genetic reductions in growth that result in a decline of harvestable biomass.

This article provides a short overview of research that has been carried out to investigate the prevalence and consequences of fisheries-induced adaptive changes in exploited marine species using the maturation reaction norm approach.

#### 2.4.2 The Concept of Probabilistic Reaction

Reaction norms for age and size at maturation describe the age and size dependence of an organism's maturation process and were first introduced by Stearns and Koella (1986). Maturation is not, however, fully determined by age and size alone. The residual effects not captured by age and size introduce an unavoidable probabilistic element to the description of the maturation process. Taking this probabilistic nature of maturation into account is crucial if maturation reaction norms are to be estimated from data. The probabilistic reaction norm for age and size at maturation (Heino *et al.* 2002a) is defined as the probability that an immature individual, depending on its age and size, matures during a given time interval. A probabilistic reaction norm is thus specified by determining these probabilities for all relevant ages and sizes (see Figure 2.4.2.1).

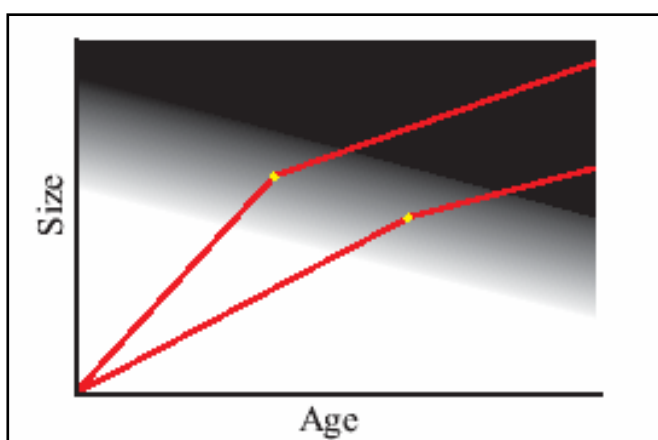


Figure 2.4.2.1 Probabilistic reaction norms for age and size at maturation describe how the probability of an organism maturing during a given time interval depends on its age and size. Shades of grey illustrate how this probability may increase with age and size. Two growth trajectories are shown in red, one for a slow-growing fish and one for a fast-growing fish. Yellow dots indicate the age and size at which these fish mature.

The traditional way of describing maturation in fisheries science is based on so-called maturity ogives. These depict the proportions of mature individuals in a population as functions of age and/or size. However, it is crucial to realize that maturity ogives characterize only the maturity status of a stock, and not the maturation process itself. This is because the maturity status of a population is determined not only by the maturation process itself but also by growth and survival. When analyzing trends in maturity ogives, changes in the maturation process are thus not distinguishable from changes in the rates of growth and/or mortality. All three factors are affected by fishing, and it is therefore very difficult, if not impossible to use maturity ogives for building an understanding of how specifically exploitation impacts a stock. Maturation reaction norms, by contrast, largely overcome these difficulties: by describing the maturation process itself (in terms of the effects of age and size) they strip away the confounding effects of varying growth and survival. This property of maturation reaction norms facilitates addressing important research problems, including:

- Disentangling phenotypically plastic and genetic changes in maturation, and



- Predicting how maturation is influenced by changes in growth and/or mortality.

Both of these tasks are central to assessing, understanding, and predicting the population characteristics of marine organisms. Maturation reaction norms also allow the effects of variations in factors other than growth and mortality to be studied. This is because many variables, such as food availability and temperature, influence maturation mostly through their effects on growth and survival.

The specific methodology for carrying out estimations of maturation reaction norms for several types of commonly available data is now available (Heino *et al.* 2002a, b; Barot *et al.* 2004a). While applications of this new methodology have so far utilized data from commercially exploited fish stocks, the approach is readily applicable to all sorts of organisms, and even to ontogenetic transitions other than maturation.

### 2.4.3 Applying the Probabilistic Maturation Reaction Norm Approach to Case Studies

To date, probabilistic maturation reaction norms have been estimated for eleven marine stocks and one freshwater stock of exploited fish (see Table 2.4.3.1).

Table 2.4.3.1. Literature survey of empirical case studies of fisheries-induced evolution in maturation reaction norms.

Stock	Period with data	Reference
Northeast Arctic cod	1932–1998	Heino <i>et al.</i> 2002b
Georges Bank cod	1970–1998	Barot <i>et al.</i> 2004b
Gulf of Maine cod	1970–1998	Barot <i>et al.</i> 2004b
Northern cod	(1977)–1981–2002	Olsen <i>et al.</i> 2004
Grand Bank cod	1971–2002	Olsen <i>et al.</i> 2004
St. Pierre Bank cod	1972–2002	Olsen <i>et al.</i> 2004
North Sea plaice	1955–1995	Grift <i>et al.</i> 2003
Newfoundland plaice	1973–1999	Barot <i>et al.</i> 2004c
Grand Bank plaice	1969–2000	Barot <i>et al.</i> 2004c
St. Pierre Bank plaice	1972–1999	Barot <i>et al.</i> 2004c
Atlantic herring	1930–1992	Engelhard and Heino 2004
Norwegian grayling	20th century	Haugen and Vøllestad 2004

All but one of the investigated stocks exhibit clear temporal trends in their maturation reaction norms. These always occurred towards increased maturation probabilities at younger ages and smaller sizes, in accordance with predictions from life-history theory. In the Canadian cod stocks, there are tentative signs for a reversal of this trend, resulting from the moratorium declared on directed offshore cod fishing in 1992. The one exception in an otherwise overwhelmingly clear pattern of evolutionary changes is the Atlantic herring stock, where maturity changes have been predominantly plastic.

### 2.4.4 Theoretical Studies of Fisheries-induced Evolution of Maturation Reaction Norms

Two theoretical studies have investigated fisheries-induced evolution in maturation reaction norm. First, Ernande *et al.* (2004) studied a generic model for the evolution of deterministic maturation reaction norms in harvested populations. This study is based on adaptive dynamics theory, an approach particularly suited for describing phenotypic evolution in complex ecological settings. This is the first study in which adaptation to a heterogeneous environment is not trivially deducible from adaptations to the underlying environmental components.

Second is an ‘eco-genetic’ approach developed by Dieckmann and Heino (2004) that includes both a process-oriented ecological description of fish population dynamics and a quantitative genetic component for characterizing the distribution of reaction norm parameters in a population. This model allows predicting the direction and pace of evolutionary changes in maturation reaction norms in response to fisheries-induced selection. In addition, the model can be used to predict the phenotypic and demographic responses of a stock to changes in its environment, e.g., in growth or mortality rates. A version of this model has been parameterized for Northeast Arctic cod; it indicates that both the time scale and the magnitude of changes documented in the Northeast Arctic cod’s maturation reaction norm are well in accordance with what is expected based on changes in the cod’s exploitation regime. One especially important result of this study is an asymmetry observed in the rates of evolutionary changes: evolutionary recovery of age and size at maturation appears to be 5 to 10 times slower than detrimental fisheries-induced evolutionary changes, implying that for each additional year of current fishing regime 5 to 10 additional years will be needed for evolutionary recovery.

#### 2.4.4.1 Estimating Quantitative Genetic Parameters of Maturation Reaction Norms

Linking the probabilistic maturation reaction norm approach to quantitative genetics is important for confirming the genetic basis of observed temporal trends in maturation reaction norms and for assessing the relative role of environmentally influenced factors other than growth. Additionally, such a link will provide the necessary estimates of quantitative genetic parameters for predicting future fisheries-induced evolution of maturation reaction norms. Notably, this would allow a better evaluation of the consequences of management scenarios for the genetic recovery of maturation tendency in exploited fish stocks.

The distribution of age and size at maturation can be inferred from a probabilistic maturation reaction norm and analysed with methods of quantitative genetics. Variance-covariance components of maturation can then be decomposed using different descriptions of the phenotype. First, one can focus on a bivariate phenotype comprising age and size at maturation,  $(a_m, s_m)$ , and apply a model of classical quantitative genetics (Lynch and Walsh 1998). Such a model decomposes phenotypic (co)variance for age and size at maturation into genetic (co)variance, growth-related (co)variance, macro-environmental (co)variance unaccounted by growth, and interactions (co)variances. Alternatively, instead of focusing on a bivariate phenotype, one can view the phenotype of individuals as being given by the infinite collection of phenotypic components describing age and size at maturation for a specific growth rate. This can be considered as an extension of the multivariate phenotype as introduced by Lande (1979) and is related to the work on the quantitative genetics of infinite-dimensional, or function-valued, traits that has been developed by Kirkpatrick and co-workers (Kirkpatrick and Heckman 1989; Kirkpatrick *et al.* 1990; Gomulkiewicz and Kirkpatrick 1992; Kirkpatrick and Lofsvold 1992):

- Bivariate perspective:  $(a_m, s_m)$ ,
- Multivariate perspective:  $((a_{mg_1}, s_{mg_1}), \dots, (a_{mg_i}, s_{mg_i}), \dots, (a_{mg_n}, s_{mg_n}))$ ,
- Infinite-dimensional perspective:  $(a_m[g], s_m[g])$ ,

with  $g_i$  denoting growth rate in the multivariate case and  $g$  denoting growth rate in the infinite-dimensional case. For maturation reaction norms, the dependence on environment, i.e., growth, can also be avoided altogether: the reaction norm is then simply described as  $s_m[a]$ . Again, the phenotypic (co)variance of phenotypic components, i.e., of the points along the reaction norm  $s_m[a]$ , comprises genetic, environmental, and interaction (co)variances. These are no longer described by matrices but by functions, which describe the (co)variance between two specific phenotypic components,  $s_m[a]$  and  $s_m[a']$ . In this case, the environmental effect no longer contains the effect of growth, since the maturation reaction norm is constructed such as to be independent of growth.

In order to complete the decomposition of phenotypic (co)variance, the coefficient of relatedness between studied individuals must be known. Classically, such information is obtained by performing controlled mating experiments combined with multi-environment rearing. Such quantitative genetics experiments allow an accurate estimation of (co)variance components. However, commercially exploited fish are often late-maturing species, thus requiring long-term experiments. Additionally, experimental rearing conditions are unlikely to be representative of natural environmental variation. An alternative approach would be to estimate relatedness between wild individuals using molecular markers (see for review *Awise et al.* 2002; *Wilson and Ferguson* 2002; *Jones and Ardren* 2003). This approach allows to properly account for natural environmental variation and to avoid long experiments. Presently, there has been very little empirical work that applies marker-inferred relatedness to quantitative genetic studies on wild fish populations. Effective population sizes of most commercial fish species are so large that relatedness between sampled individuals is often not significantly different from 0. In addition, the number of loci necessary for estimating relatedness increases with effective population size (*Jones*, 2003). Hopefully, molecular estimates of relatedness can be used under semi-natural experimental conditions. In situ applications to marine and large freshwater habitat might be used in species with tendencies towards low dispersal or philopatry such as salmonids. Special attention should be given to species and/or populations exhibiting small effective population size, in which relatedness between individuals is more likely to be significantly different from 0.

These links between the reaction norm approach and quantitative genetics are currently in the process of being developed. Future research will need to strengthen this bridge.

#### 2.4.5 Conclusions

Recent empirical insights, based on the method of probabilistic maturation reaction norms, have confirmed that fisheries-induced evolution is occurring in nature. Many long-term stock assessment datasets available worldwide are still waiting to be analyzed. Monitoring and management routines, including suitable reference points, will need to be developed to account for this additional threat to exploited stocks. The WG endorses the use of probabilistic maturation reaction norms as a tool for evaluating the evolutionary and genetic effects of selective fishing.

#### 2.4.6 Recommendations

The WG recommends additional scientific investigations on:

- a) assessing fisheries-induced evolution in adaptive traits other than maturation, including growth rates, reproductive effort, skipped spawning, and behaviour;
- b) measuring quantitative genetics parameters of exploited stocks needed for predicting the expected pace of fisheries-induced evolution;
- c) clarifying the mechanisms that link fisheries-induced evolution to decreases in stock stability, yield, and recovery potential;
- d) investigating how fisheries regimes that are least detrimental for genetic composition depend on the life-history patterns of exploited stocks;
- e) developing appropriate management and assessment tools for coping with fisheries-induced evolution.

*It is recommended that ICES sponsor an international symposium on fisheries-induced evolution.*

Such an event will serve as a platform for integrating recent scientific advances, facilitating the lines of future research summarized above, and for initiating development of the practical tools that will be needed in the future for the assessment and management of fisheries-induced evolution.

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## 2.5 Commence work on a list of species for which there is reason to be concerned for loss of genetic variation, and a list of species for which there is good genetic information from which to advance management advice (ToR e).

This section is based on a position paper prepared by H. Wilcock, G. Carvalho, G. Dahle, H. Knutsen, K. Roth and E. Kenchington and adopted by the WGAGFM in Hamburg in 2004.

### 2.5.1 Response to the ToR

The ICES Working Group on the Ecosystem Effects of Fisheries (WGECO) requires information on the availability of genetic information in order to make further progress on the development of advisory forms appropriate to the preservation of genetic diversity of exploited stocks and stocks suffering substantial mortality as bycatch. The WG was tasked with commencing work on a species list for which genetic information could be used to advance management advice. As this list could be quite extensive, the WG began by constraining our list to meet certain criteria. In compiling this information we have only included data on commercial marine species from the North Atlantic and adjacent seas. We have included only those species where structuring has been detected and where we feel that there is a reliable case supporting the subdivision(s). There are many studies which have demonstrated genetic homogeneity across large areas, but many of these might have found more structuring with more rigorous sampling, alternative genetic markers and/or data analysis.

We consider that sufficient genetic data which could be incorporated into management decisions are available for 21 commercial species (Table 2.5.1.1). This list is not exhaustive and absence from the list is not meant to imply that genetic data are not available for that species, nor that management advice could not be extracted from other publications. The WG interpreted the ToR such that this preliminary list would be the starting point for a joint consideration of current management practices with known information on the genetics of these species/stocks. Consequently, we focused on species for which we felt that there *may be* discordance between current management practices and the genetic data.

The genetic data available on these species mainly originate from studies focusing on population genetics, and the majority of studies have been concentrating on the “major” commercial species i.e. cod, herring and haddock (Table 2.5.1.2). For the most part, these genetic studies only deal with neutral genetic variation collected with microsatellite DNA primers or with mitochondrial DNA markers. These population genetic data could however be utilised by fisheries management to define stock structure and/or commercial units. Table 2.5.1.2 provides an annotated list of some of the studies which could be used to provide advice for the species in Table 2.5.1.1. There are very few studies on adaptive variation which is an important aspect (and perhaps most important) to consider in management, although a number of “projects in progress” were known to the WG and more information on this aspect should be available in the near future.

Table 2.5.1.1. A list of commercial species in the ICES North Atlantic for which genetic data are sufficient to incorporate into management advice.

Species	Common Name (s)
<i>Melanogrammus aeglefinus</i>	Haddock
<i>Gadus morhua</i>	Cod
<i>Scomber scombrus</i>	Atlantic mackerel
<i>Merluccius merluccius</i>	European hake
<i>Clupea harengus</i>	Atlantic herring
<i>Dicentrarchus labrax</i>	European Sea bass
<i>Engraulis encrasicolus</i>	European anchovy
<i>Thunnus obesus</i>	Bigeye tuna
<i>Thunnus thynnus</i>	Bluefin tuna
<i>Solea solea</i>	Common sole
<i>Micromesistius poutassou</i>	Blue whiting
<i>Melanogobius merlangus</i>	Whiting
<i>Anguilla anguilla</i>	European eel
<i>Homarus gammarus</i>	European lobster
<i>Ostrea edulis</i>	European flat oyster
<i>Mytilus edulis/trossulus/galloprovincialis</i>	Mussels
<i>Xiphias gladius</i>	Swordfish

<i>Pleuronectes platessa</i>	Plaice
<i>Scophthalmus maximus</i>	Turbot
<i>Pomatochistus minutus</i>	Goby
<i>Pomatochistus lozanoi</i>	Goby

The impacts of fishing on genetic diversity are just beginning to be quantified (see ToR d, and Kenchington 2003). In Table 2.5.1.3 we have compiled a global list of extant species for which there is direct evidence for a loss of genetic diversity due to exploitation. This list includes only one example from the North Atlantic. This type of analysis requires comparison of historical and contemporary samples with the same markers. In many cases historical data or archived samples are not available, so we may never be able to assess loss of genetic diversity associated with human activities for many species. For this reason, collections of otoliths, scales and tissue from historical time periods are extremely valuable.

Table 2.5.1.3. A compilation of species for which a loss of genetic variation has been attributed to exploitation.

Species	Common Name	Geographic Area	Genetic Impact	Reference
<i>Pagrus auratus</i>	New Zealand snapper	New Zealand	Significant decline in genetic diversity during exploitation history (1950 – 1998)	Hauser <i>et al.</i> 2002
<i>Gadus morhua</i>	Cod	North Sea	Detection of decline in genetic diversity associated with period of heavy exploitation. Suggestion of replacement of a declining population via increased effective immigration.	Hutchinson <i>et al.</i> 2003
<i>Hoplostethus atlanticus</i>	Orange roughy	New Zealand	Loss of genetic diversity associated with fishing pressure	Smith <i>et al.</i> 1991
<i>Cephalorhynchus hectori</i>	Hector's dolphin	New Zealand	Comparison of historical and contemporary samples showed significant loss of genetic diversity associated with fisheries related mortality	Pichler and Baker 2000

The WG was also asked to consider stocks suffering substantial mortality as bycatch. Bycatch of commercial and non-commercial species may be imposing genetic changes in natural stocks or populations in the same way as selective fishing (see ToR d) appears to be affecting target species. At present there is no work dealing with genetic effects or changes due to large incidents of bycatch removal but this aspect of selection could be examined using a genetic approach. Bycatch of non-target species including benthic epifauna, could potentially be a major problem, introducing unknown effects on the ecosystems, including reducing the habitats (i.e. spawning and nursery areas) for other marine species. Henry and Kenchington (2004) have observed genetic differences in *Sertularia cupressina*, a colonial benthic hydroid, which they attribute in part to the impact of mobile gear on the benthos. To our knowledge, this is the only study which has examined the genetic impacts of fishing on non-target species.

Additionally, bycatch of early life history stages of all species, including commercial species has been overlooked as having a potential impact on the populations. Halibut, turbot, sole and plaice larvae caught in shrimp trawling, and young coastal cod caught in eel-traps constitute examples whereby bycatch removal might change the overall composition of the different adult populations. The prohibition of shrimp trawling in some areas might be the reason for the observed increasing abundance of 3–4 kg halibut caught on gill-net in the coastal area of Norway and it would be interesting to apply genetic approaches to these questions, although it is unlikely that fishing selection on the larval and juvenile stages will have a genetic impact on adult populations.

## 2.5.2 Recommendations

Historical collections of commercial and other species are of extreme value in assessing loss of genetic diversity due to anthropogenic effects. These should be given a high priority for preservation and made available for genetic research.

Careful analysis of genetic and phenotypic variation should be made and integrated into physical oceanographic and ecological knowledge and modelling in stock assessments.

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Table 2.5.1.2. A semi-annotated list of commercial species in the North Atlantic and adjacent seas for which genetic information has shown population structuring.

Species	Common Name (s)	Geographic Area	Type of Genetic Information	References
<i>Melanogrammus aeglefinus</i>	Haddock	Northwest Atlantic Georges Bank	Microsatellite data Georges Bank sample relatively stable in genetic diversity over 40 year period BUT discrete spawning stocks identified in NW Atlantic, esp. Nantucket shoals Stocks from NW Atlantic are distinct from Grand Banks/ eastern Atlantic mtDNA data Limited gene flow and discrete stocks in the western North Atlantic	Lage <i>et al.</i> 2001    Zwanenburg <i>et al.</i> 1992
<i>Gadus morhua</i>	Cod	UK waters  Northwest Atlantic Newfoundland/ Labrador	Microsatellite data Marked genetic structure around UK, including 4 distinct populations within the North Sea Strong differentiation of Barents Sea from all UK sites Microsatellite data and PanI Distinct offshore spawning populations detected, inshore stocks more homogeneous Microsatellite data Long term stability of genetic composition of offshore cod aggregations Differences between inshore and offshore cod Small scale population structure Differences in north-west Atlantic cod at continental and spawning bank scales Large scales: populations differentiated by topographical features e.g. submarine trenches Small scales: Differences associated with oceanographic and spatio-temporal spawning patterns Regional differences detected Two distinct populations in the North Sea and Baltic Hybrid zone detected between two seas, zone divides distinct populations Detection of weak but significant genetic differences between sites within 300km range. Suggests fine-scale population structure of spawning stocks Identification of spatially and temporally distinct larval aggregations. Important information for examination of reproductive success and recruitment Long distance transport of cod larvae	Hutchinson <i>et al.</i> 2001    Beacham <i>et al.</i> 2002  Ruzzante <i>et al.</i> 1997, 1999, 2000, 2001  Ruzzante <i>et al.</i> 1998   Ruzzante <i>et al.</i> 2000 Nielsen <i>et al.</i> 2001 Nielsen <i>et al.</i> 2003 Knutson <i>et al.</i> 2003 Ruzzante <i>et al.</i> 1996 Knutson <i>et al.</i> 2004



Species	Common Name (s)	Geographic Area	Type of Genetic Information	References
<i>Scomber scombrus</i>	Atlantic mackerel	Eastern Atlantic	MtDNA data D loop and Cyt B Distinct Eastern and Western Atlantic stocks Eastern Atlantic mackerel divided into three stocks and should be managed separately.	Nesbø <i>et al.</i> 2000
<i>Merluccius merluccius</i>	European hake	Europe and Mediterranean  Bay of Biscay (Cape Breton Canyon)  Europe and Mediterranean	Microsatellite data Sub-structuring within Atlantic samples suggesting current management units not suitable Confirms structuring between Atlantic and Mediterranean samples Microsatellite data Canyon did not act as barrier to gene flow Temporal instability of genetic patterns suggests recruitment variation Allozyme data Genetic structuring between Atlantic and Mediterranean samples Some indication of structuring within Atlantic	Lundy <i>et al.</i> 1999   Lundy <i>et al.</i> 2000  Roldan <i>et al.</i> 1998
<i>Clupea harengus</i>	Atlantic herring	Norwegian Sea, Barents Sea  Northwest Atlantic	Microsatellite data Significant differences between Norway and Barents Sea also between spawning groups Coastal populations showed significant population structure	Shaw <i>et al.</i> 1999  McPherson <i>et al.</i> 2001
<i>Dicentrarchus labrax</i>	European sea bass	Mediterranean  Atlantic/ Mediterranean  Mediterranean	Microsatellite data Evidence for genetic differences between Spanish and France populations Strong genetic divide around Atlantic/ Med. Transition area across short distance. Weaker evidence for differentiation amongst Atlantic populations Significant differentiation between eastern and western Med Eastern basin further divided into Adriatic, Ionian, Lybico-Tunisian Gulf and Aegean seas Differentiation probably sufficient to be different spawning groups Microsatellite and Allozyme data Differentiation between lagoon and marine samples detected	García de León 1997  Naciri <i>et al.</i> 1999 Allegrucci <i>et al.</i> 1997 Bahri-sfar <i>et al.</i> 2000  Lemaire <i>et al.</i> 2000
<i>Engraulis encrasicolus</i>	European anchovy	Adriatic  Mediterranean	Allozyme data and morphometric Evidence for two distinct stocks separated into NW and SouthCentral areas mtDNA data Significant geographic heterogeneity	Bembo <i>et al.</i> 1996  Bembo <i>et al.</i> 1996

Species	Common Name (s)	Geographic Area	Type of Genetic Information	References
<i>Thunnus obesus</i>	Bigeye tuna	Worldwide	mtDNA data Atlantic and Indian Ocean form distinct stocks	Chow <i>et al.</i> 2000
		Worldwide	mtDNA data Atlantic and Indian Ocean form distinct stocks	Bremer <i>et al.</i> 1998
<i>Thunnus thynnus</i>	Bluefin tuna	North Atlantic	Allozymes, mtDNA data Limited genetic differentiation between West Atlantic and East Atlantic/Mediterranean	Pujolar <i>et al.</i> 2003
<i>Solea solea</i>	Common sole	North East Atlantic and Mediterranean	Allozyme data Some differentiation at a regional scale and isolation by distance between Mediterranean and Atlantic	Kotoulas <i>et al.</i> 1995
		North East Atlantic	Distinct genetic differentiation detectable between European stocks (N/S cline) RAPD data Isolation by distance and clustered in 2 groups: continental Europe and British Isles	Exadactylos <i>et al.</i> 1998 Exadactylos <i>et al.</i> 2003
<i>Micromesistius poutassou</i>	Blue whiting	North East Atlantic/Mediterranean	Allozyme data Partially differentiated stocks in Med. and eastern Barents Sea	Gjaever and Stein 1998
<i>Melanogaster merlangus</i>	Whiting	North Atlantic	Microsatellite data Significantly different allele frequency distributions found between Borgensfjord and northern and southern North Sea samples	Rico <i>et al.</i> 1997
<i>Anguilla anguilla</i>	European eel	North East Atlantic/Mediterranean	mtDNA and Allozyme data Structuring (isolation by distance) was reported by Wirth and Bematchez but there are concerns over these results (see ToR c)	Wirth and Bematchez 2001, 2003
		North East Atlantic	Isolation by distance but concerns over these results (see ToR c)	Maes and Volckaert 2002
	Iceland North East Atlantic/Mediterranean and Baltic	Iceland	Hybridisation in Icelandic waters between American and European eels	Avise <i>et al.</i> 1990
			Microsatellite data Stronger temporal than spatial segregation	Dannewitz 2003
<i>Homarus gammarus</i>	European lobster	Norway	Microsatellite and mtDNA data Structuring was reported among populations in northern Norway	Jørstad <i>et al.</i> 2004
<i>Epinephelus marginatus</i>	Dusky grouper	Mediterranean	Microsatellite and Allozyme data Structuring among populations but no evidence for isolation by distance	De Innocentiis <i>et al.</i> 2001
<i>Xiphias gladius</i>	Swordfish	Atlantic Europe	mtDNA data Differences between Mediterranean and Atlantic populations	Kotoulas <i>et al.</i> 1995

Species	Common Name (s)	Geographic Area	Type of Genetic Information	References
<i>Ostrea edulis</i>	European flat oyster	Atlantic Europe and Mediterranean	Allozyme and Microsatellite Data Differentiation among populations and isolation by distance	Launey <i>et al.</i> 2002
<i>Pleuronectes platessa</i>	Plaice	Iceland and North Sea	Microsatellite data Differences between regions found	Hoarau <i>et al.</i> 2002
<i>Scophthalmus maximus</i>	Turbot	North East Atlantic and Baltic	Microsatellite data Significant differentiation and sharp cline between North Sea and Baltic	Nielsen <i>et al.</i> 2004
<i>Pomatoschistus minutus</i>	Goby	North Sea	Microsatellite data Small scale differences in the Southern Bight of the North Sea Allozyme data Small scale differences comparing inshore and offshore	Pampoulie <i>et al.</i> in press Gysels <i>et al.</i> in press
<i>Pomatoschistus lozanoi</i>	Goby		Allozyme data Small scale differences comparing inshore and offshore	Gysels <i>et al.</i> in press
<i>Mytilus edulis/trossulus/galloprovincialis</i>	Mussels	Worldwide	Allozyme data and other	Various

### 3 Working Group business

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#### 3.1 Draft resolutions 2005 and suggestions for ToR and meeting place in 2006

During discussions on meeting place in the year 2005, the WG responded positively to a generous invitation from Dr Einar Eg Nielsen, Danish Institute for Fisheries Research, Silkeborg, Denmark, to host the 2005 WG meeting from 3–6 May 2005. The 2006 meeting is provisionally planned for Newport, Ireland, at the invitation of Dr Philip McGinnity. It was agreed that future meetings should plan for 3.5 days of business in order to allow for a full discussion of the ToRs. Over the past few years the ToRs are increasing in complexity and require more time for discussion in order to reach consensus on the report.

#### Draft resolutions 2005

Concerning Terms of Reference and meeting place for the year 2005, WGAGFM in plenary recommended that:

The **Working Group on the Application of Genetics in Fisheries and Mariculture** (Chair: Dr E. Kenchington, Canada) proposes to meet in Silkeborg, Denmark, 3–6 May 2005 to:

- a) review information on the nature and rates of environmental change as well as key factors to determine the evolutionary ability of fish stocks to respond to climate change (lead P. McGinnity (Ireland));
- b) review of methods for, and application of mixed-stock and assignment analysis for the elucidation of stock components, with an emphasis on marine fishes, and provide recommendations for applications in different species and types of fisheries (lead D. Bekkevold (Denmark));
- c) review methods and evidence for elucidating local adaptation in marine fishes (lead G. Carvalho (UK));
- d) evaluate the usefulness of probabilistic maturation reaction norms as ecological quality objectives (EcoQOs) as an early warning signal for the negative impact of fishing and other anthropogenic activities (lead P. McGinnity (Ireland) with support from U. Dieckmann (Austria) and B. Ernande (France));
- e) investigate possible genetic erosion and changes in life history characteristics of local stocks due to mariculture activity (lead G. Dahle (Norway)).

Priority:	WGAGFM is of fundamental importance to the ICES advisory process and address a variety of questions raised in the ICES Strategic Plan.
Scientific Justification and Relation to Action Plan:	<p>2.5, 2.6, 2.12, 3.4, 3.7, 3.9, 4.7</p> <ol style="list-style-type: none"><li>a) The Potential Impact of Climate Change on Fisheries. Significant progress has been made in modelling past environments and predicting likely future marine climate states. Fish stocks will respond in yet unpredictable ways to such changes. Consideration of such responses will include potential impacts on distribution and abundance, as well as evolutionary capacity. Part of these considerations will include reference to contemporary anthropogenic-induced direct and indirect genetic change as a result of selective fishing, introgression, habitat change, pollution, aquaculture activity. This exercise will yield information on the nature and rates of environmental change as well as key factors to determine the ability of fish stocks to respond to this change.</li><li>b) Fisheries management should maximize the sustainability of individual stock components. Many fisheries take place on mixed populations and this complicates management of individual population/stock components, and leads to risk of overexploiting smaller population components. Genetic mixed-stock analysis and assignment methods provide a means for disentangling complex stock structures and their temporal variability. Individual methods vary in their properties for identifying mixed stocks and estimating</li></ol>

	<p>stock proportions in species exhibiting high gene flow, such as is characteristic of many marine fishes. We propose to provide a review of the available estimation methods, their application to date, and of advantages and disadvantages of applying different methods to different stock structures.</p> <p>c) Although it is widely accepted that fish stocks exhibit marked phenotypic variation in many traits, little is known of the genetic basis of such variability. The development of new markers and statistical theory has facilitated opportunities for estimating the nature and extent of the genetic component of this variation. This ToR will consider the range of options most appropriate for detecting local adaptation in marine fishes, including aspects of experimental design, applications of genomic technologies and quantitative genetic approaches. Such considerations will facilitate conservation of population diversity, more effective incorporation of population heterogeneity into stock assessment models and the translation of this information into management advice.</p> <p>d) The successful application of the ecosystem approach to fisheries management will require empirical (defendable) diagnostic metrics that can detect changes in critical processes due to fishing that in turn affect key population characteristics and that ultimately determine population demographics (population size, biomass, rates of extinction, (persistence) etc.), life history or quantitative variability and evolutionary (genetic) potential, particularly with regard to climate change and disease challenges. The development or identification of these metrics has to date proved problematic within WGECO, the ICES working group tasked with this objective. New research undertaken by Ulf Dieckmann, Bruno Ernande and co-workers working with probabilistic reaction norms and discussed within WGAGFM and in a theme session at the ICES ASC in 2002, provides a realistic opportunity for the application of this method as an EcoQO. It is recommended here that the WGAGFM summarize the current application and build on its 2004 ToR with a view to establishing reference limits for the react norms and apply them to other factors causing selection variance such as disease, to evaluate their broader applicability as an EcoQO. We propose to take our results to the WGECO for their subsequent evaluation.</p> <p>e) Increasing mariculture activity, including species such as cod, halibut and mussels, may impose genetic changes on local stocks which could translate to changes in time of spawning, spawning behaviour etc., with ecosystem-wide consequences. We propose to evaluate these interactions with two case studies, cod and the pearl oyster for which we believe sufficient information exists to assess this impact from a genetic perspective.</p>
Relation to Strategic Plan:	Responds to Objectives 1.3 (a), 1.10 (b,c), 2.5, 4. (d), 2.6, 3.11 (e)
Resource Requirements:	None required other than those provided by the host institute.
Participants:	WGAGFM members, invited contributors and observers
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	ACME, ACE
Linkages to other Committees or Groups:	SIMWG (Delegates drew specific attention to the need to develop this link – the Chairs of these two Working Groups should correspond together to ensure that there is no unnecessary overlap in their work.)
Linkages to other Organisations:	None in the current year

### **3.2 Other business**

The WG discussed and endorsed a proposal brought forward by F. Volckaert (Belgium) for a half or full day theme session at the 2006 ICES ASC in Scotland. Sponsorship of the session, including travel costs for keynote and invited speakers would be provided by the EU through a presently funded Marine Genomics network. The WG further endorsed a proposal from U. Dieckmann (Austria) and detailed in the recommendations for ToR d to pursue an ICES sponsored symposium on Fisheries Induced Evolution.

P. Boudry announced that the next Genetics in Aquaculture meeting would be held in Montpellier, France in 2006 and encouraged the WG to use this as an opportunity to advertise ourselves and our activities. It was agreed that WGAGFM should prepare a poster for display at this meeting.

### **3.3 Adjournment of the meeting**

The meeting was adjourned at approximately 1600 on 5 May, 2004 with all recommendations discussed in plenary. It was agreed that ToR a) would be finalized through correspondence following the meeting to allow time for some critical input. The final text was circulated to all meeting participants and forwarded to the Chair of the Mariculture Committee for review.

## 4 Annexes

### Annex 1 Terms of reference 2004

2F04 The **Working Group on the Application of Genetics in Fisheries and Mariculture** [WGAGFM] (Chair: E Kenchington, Canada) will meet in Hamburg, Germany, from 3–5 May 2004 to:

- a) provide recommendations on the applications for the estimation of effective population size in wild populations of marine fish and shellfish;
- b) evaluate the management recommendations for Atlantic salmon, developed by the SALGEN EU project;
- c) consider conservation genetics aspects required for conservation targets for eels;
- d) evaluate the use of reaction norms to evaluate the genetic impact of selective fishing;
- e) commence work on a list of species for which there is reason to be concerned for loss of genetic variation, and a list of species for which there is good genetic information from which to advance management advice.

WGAGFM will report by 31 May 2004 for the attention of the Mariculture and Diadromous Fish Committees, ACME, and ACFM.

### Supporting Information

Priority:	WGAGFM is of fundamental importance to the ICES advisory process.
Scientific Justification and Relation to Action Plan:	<p>2.5, 2.6, 2.12, 3.4, 3.7, 3.9, 4.7</p> <ol style="list-style-type: none"> <li>a) Population size is the single most important factor in sustaining a high level of genetic variation within a population of a species. Population size here refers to the genetically effective population size (<math>N_e</math>), and not the number of individuals in a population (<math>N</math>). <math>N_e</math> is considered to be the most appropriate variable for assessing population viability but there is a need to review the methods and applications for inferring <math>N_e</math> and to point out their limitations. (lead E. Eg Nielsen (Denmark))</li> <li>b) SALGEN (<a href="http://www.salgen.marlab.ac.uk">www.salgen.marlab.ac.uk</a>) is a project set up to review genetic studies on Atlantic salmon and develop management recommendations for the species. WGAGFM has been asked to review and discuss the recommendations resulting from this project. (lead E. Verspoor (UK, Scotland) or intersessional group)</li> <li>c) The return rate of glass eels from the spawning ground of the European Eel (<i>Anguilla anguilla</i>) in the Sargasso Sea to the coasts of Europe and North Africa has declined dramatically. Several factors are suspected to have caused this decline. A review on the currently available knowledge on the genetic structure of the European Eel should point out the potential dangers of losing genetic diversity and lead to management recommendations. The status of the European Eel as a catadromous species has caused confusion in scientific responsibility between ICES and EIFAC (European Inland Fisheries Advisory Commission) in the past. Therefore this review is also meant to target the levels of actions to conserve the stocks in the marine and/or freshwater phase. (lead J. Trautner (Germany))</li> <li>d) As presented in Theme Session Y of the 2002 ICES Annual Science Conference in Copenhagen, recent developments of “Adaptive Dynamics Theory” have shown how the evolution of reaction norms can be modelled to evaluate the genetic impact of selective fishing. Relatively little information is available on genetic variation of reaction norms in marine organisms. However, quantitative genetics experiments, using model and/or aquaculture species, can be performed and</li> </ol>

	<p>are likely to provide valuable data for fisheries species. We will review how these two complementary approaches can be used to study the selective effect of fisheries and related issues. (lead P. Boudry (France) with B. Ernande and U. Dieckmann (Austria))</p> <p>e) WGECO requires this information to make further progress on the development of advisory forms appropriate to the preservation of genetic diversity of exploited stocks and stocks suffering substantial mortality as bycatch.</p>
Resource Requirements:	None required other than those provided by the host institute.
Participants:	Activate membership to complete terms of reference.
Secretariat Facilities:	None required
Financial:	None required
Linkages to Advisory Committees:	ACME, ACE
Linkages to other Committees or Groups:	<p>SIMWG (Delegates drew specific attention to the need to develop this link – the Chairs of these two Working Groups should correspond together to ensure that there is no unnecessary overlap in their work.)</p> <p>WGECO WGEELs should receive report</p>
Linkages to other Organisations:	HELCOM, EC
Secretariat Cost share	ICES:100%



## Annex 2: Participants at the 2004 WGAGFM meeting in Hamburg, Germany

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Ellen Kenchington (Chair)	Dept. of Fisheries and Oceans, Bedford Inst. of Oceanography, P.O.Box 1006 Dartmouth, Nova Scotia B2Y 4A2, Canada	KenchingtonE@mar.dfo-mpo.gc.ca
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